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ABSTRACT

This is one of several short-term courses developed to assist in the training of waste water treatment plant operational personnel in the tests, measurements, and report preparation required for compliance with their NPDES Permits. The Student Reference Manual provides step-by-step procedures for laboratory application of equipment operating procedures for effluent monitoring. Each lesson outlines a specific objective, description of the analysis, and the applicability of the procedure. Parameters of this course include Total Phosphorus, Chemical Oxygen Demand, Kjeldahl Nitrogen, Ammonia, Nitrates, Oil, and Grease. (CS)

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EPA-430/1-76-006

EFFLUENT MONITORING PROCEDURES: NUTRIENTS

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STUDENT REFERENCE MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER PROGRAM OPERATIONS

SE 023 378

EPA-430/1-76-006
June 1976

EFFLUENT MONITORING PROCEDURES: NUTRIENTS

This course is designed for the treatment plant operator or technician who is required to monitor effluent discharges under a National Pollutant Discharge Elimination System (NPDES) Permit, and who has had little or no previous experience in wastewater analysis.

The course includes procedures for measuring Total Phosphorus (as P), Chemical Oxygen Demand, Kjeldahl (Total) Nitrogen, Ammonia (as N), Organic Nitrogen by difference of Kjeldahl N and Ammonia N, Nitrate-Nitrite (as N), Nitrite (as N), Nitrate (as N) by difference of Nitrate-Nitrite N and Nitrite N and Oil and Grease. The course also includes procedures for related skills--using a spectrophotometer and preparing a calibration graph.

During the course, the student will perform an approved analytical procedure for each of the measurements. At the conclusion, he will be given a certificate verifying which measurements he performed in a satisfactory manner.

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
TRAINING PROGRAM

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Reference to commercial products, trade names, or manufacturers is for purposes of example and illustration. Such references do not constitute endorsement by the Office of Water Program Operations, U.S. Environmental Protection Agency.

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A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

USE OF A SPECTROPHOTOMETER

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Use of a Spectrophotometer

This operational procedure was developed by:

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ADDRESS EPA-WPO-National Training Center, Cincinnati, OH 45268

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B.S. - Chemistry

M.S. - Chemistry

1-1/2 years Industrial Chemist

4 years college Chemistry Instructor

1-1/2 years DHEW - Air Pollution Program, Chemist

4-1/2 years DI - EPA, Chemist-Instructor

EFFLUENT MONITORING PROCEDURE: Use of a Spectrophotometer

1. Analysis Objectives:

The user of the attached effluent monitoring procedure will learn how to use the Bausch and Lomb Spectronic 20 Spectrophotometer for making colorimetric measurements.

2. Brief Description of Analysis:

In the field of water pollution analysis, many determinations are based on measuring the intensity of color at a particular wavelength. In general, color is formed in the sample by some sort of preliminary treatment such as distillation or digestion, and then adding a color developing reagent. The intensity of the color formed is related to the amount of material (such as phosphorus) in the sample. As part of the analysis, color is also developed in a series of standards; in each of the standards is a known amount of the material (such as phosphorus) of interest. A calibration curve is made using the color intensities of the individual standards and the corresponding amounts of material present. The amount of material present in the sample is determined using the calibration curve. A Bausch and Lomb Spectronic 20 Spectrophotometer is an instrument used to measure the color intensities of the standards and sample. The word absorbance is associated with the words color intensity; i.e., a sample or standard which has a low color intensity will also have a low absorbance.

Source of Procedure: Spectronic 20 Spectrophotometer Operating Manual, Bausch & Lomb, Rochester, New York 14602

Mention of a particular brand name does not constitute endorsement by the U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Use of a Spectrophotometer

General Description of Equipment Used in the Process

A. Capital

1. One Bausch and Lomb Spectronic 20 Spectrophotometer
2. One manufacturer's manual for the spectrophotometer
3. Still, or other source of distilled water
4. Hotplate
5. One spectrophotometer cell - A set of cells may be used only if the cells are optically matched. One cell would be used for each solution.

B. Reusable

1. Brush (for cleaning spectrophotometer cell)
2. Laboratory apron
3. Safety glasses
4. One pen or pencil
5. Notebook or data sheet (see page 1-23) for recording data
6. Brush (for dusting spectrophotometer)
7. One 2 liter beaker
8. One 250 ml beaker
9. One glass stirring rod
10. One 2 liter glass stoppered bottle
11. One visible phototube (Bausch and Lomb catalog number 33-29-71)
12. One infrared phototube (Bausch and Lomb catalog number 33-29-72)
13. One infrared filter (Bausch and Lomb catalog number 33-29-18)
14. Ten soft tissues (for wiping the cells)
15. One plastic squeeze distilled water bottle
16. Sink or 1 liter container for rinsing solutions
17. One 1 cm cell (to fit the Spectronic 20)

C. Consumable

1. Soap
2. Sodium dichromate, $Na_2Cr_2O_7$
3. Concentrated sulfuric acid, H_2SO_4

Items A4, B7 through B10, and C1 through C3 are for cleaning the spectrophotometer cell.

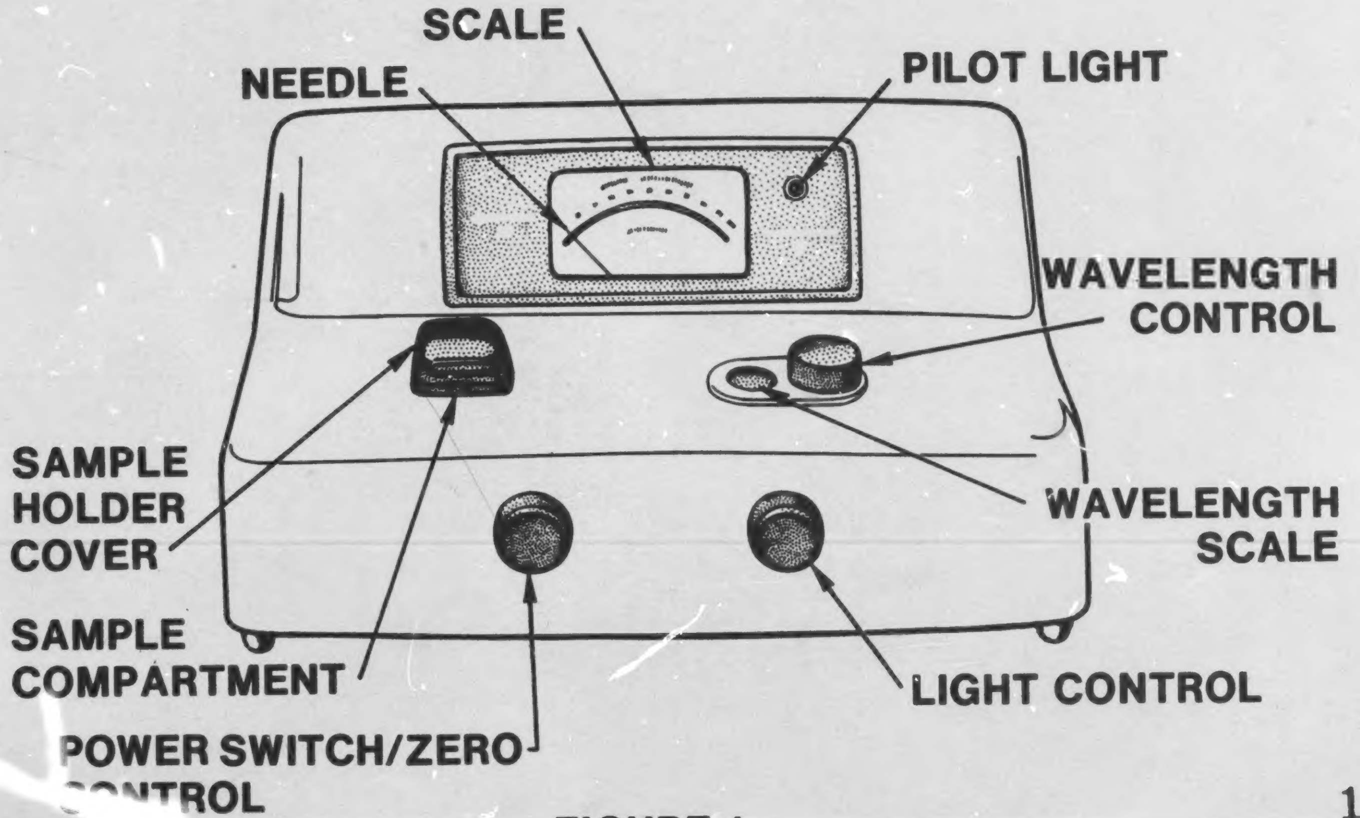

SCALE
NEEDLE
PILOT LIGHT
WAVELENGTH CONTROL
SAMPLE HOLDER COVER
WAVELENGTH SCALE
SAMPLE COMPARTMENT
LIGHT CONTROL
POWER SWITCH/ZERO CONTROL

FIGURE 1

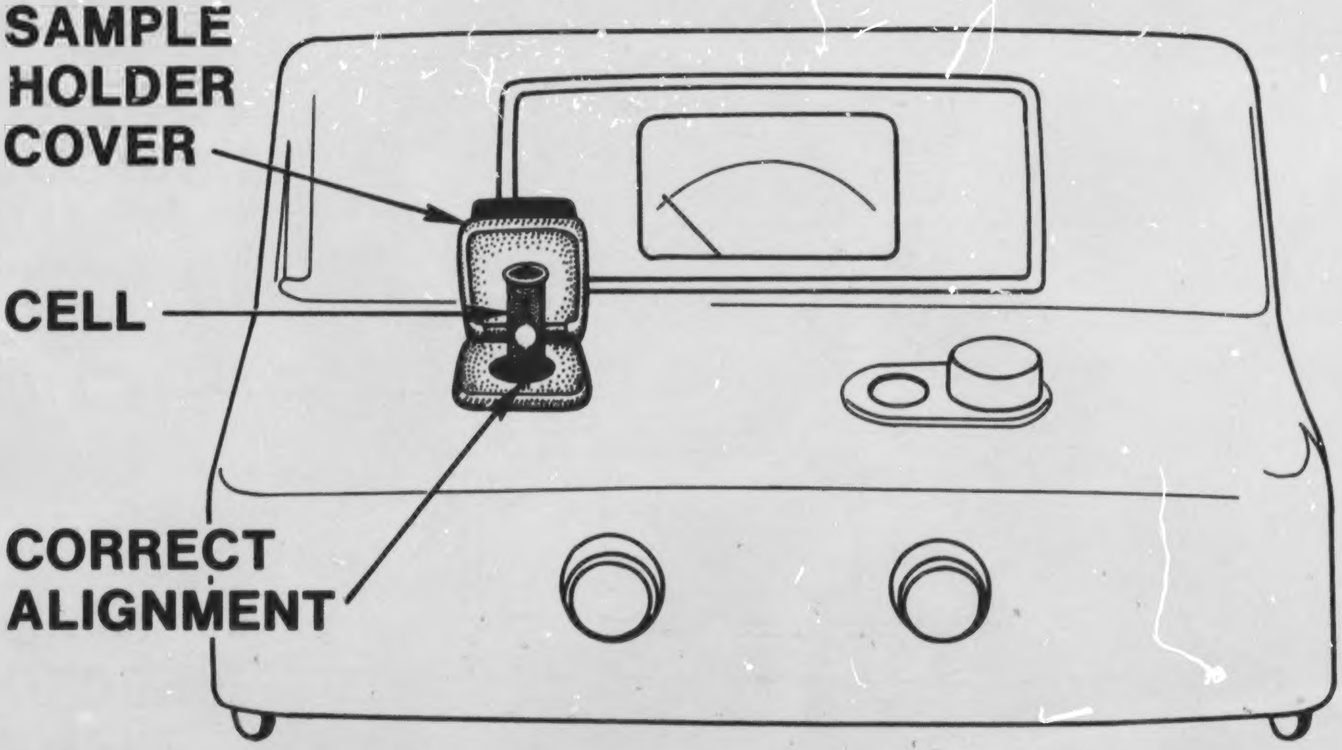

SAMPLE
HOLDER
COVER
CELL
CORRECT
ALIGNMENT

FIGURE 2

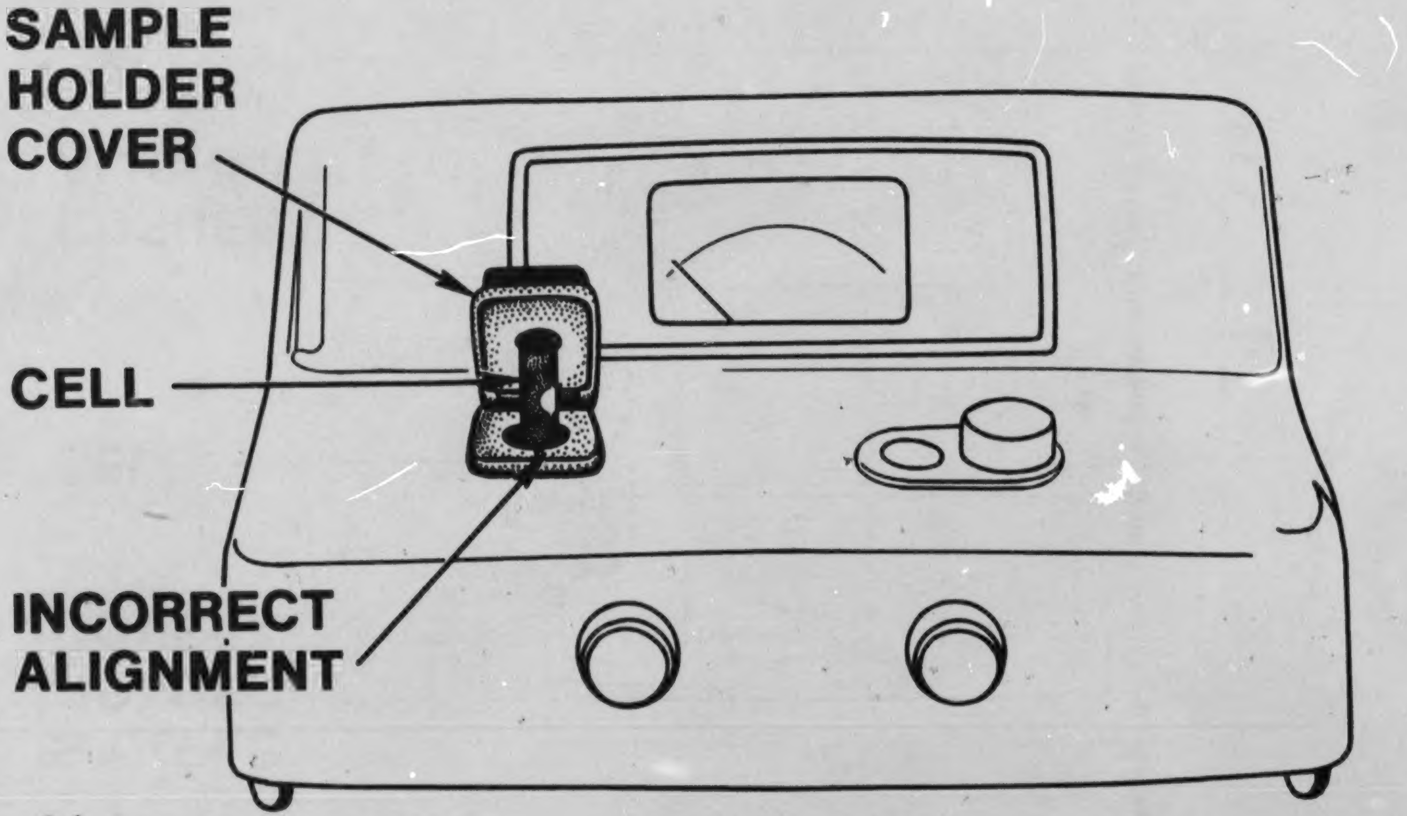
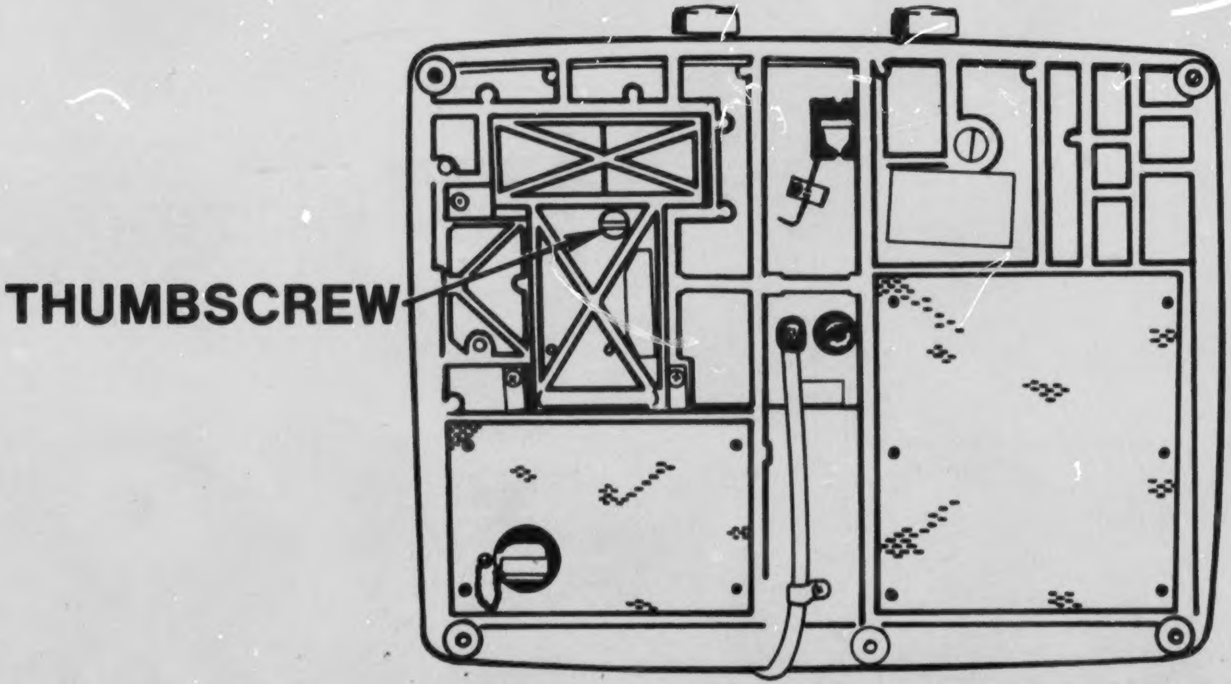
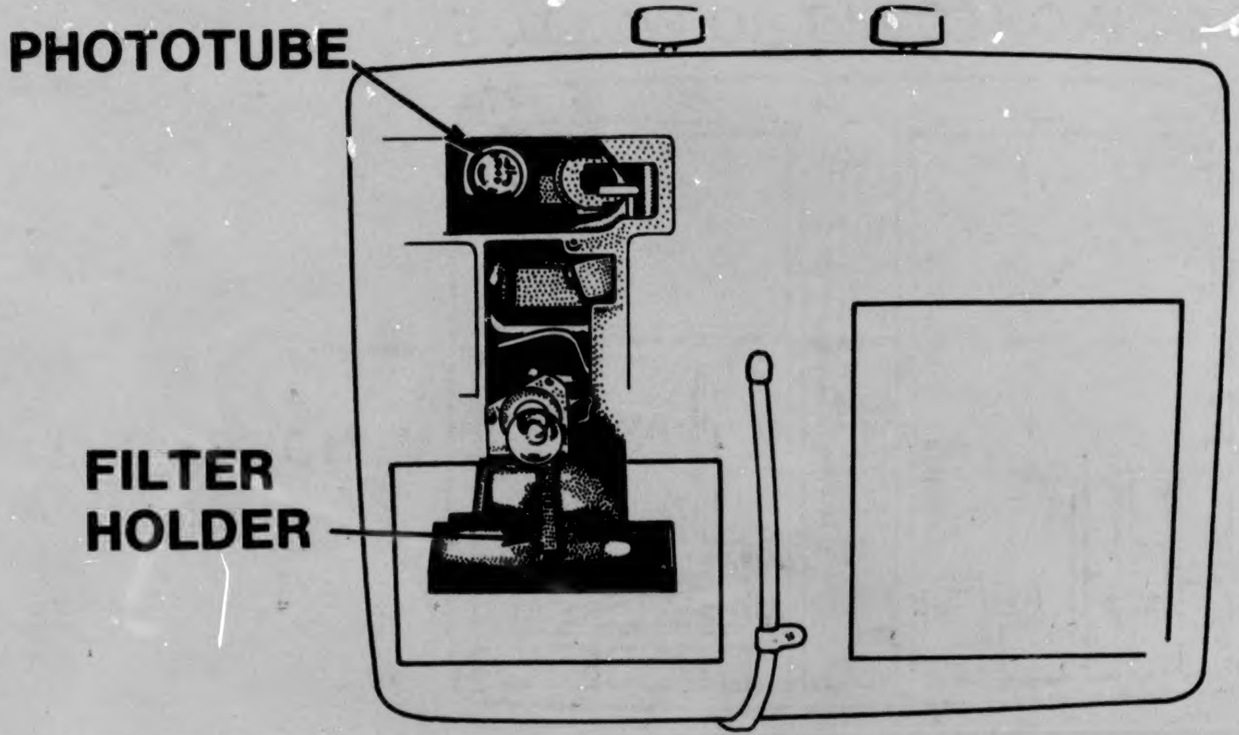

SAMPLE HOLDER COVER
CELL
INCORRECT ALIGNMENT

FIGURE 3


THUMBSCREW

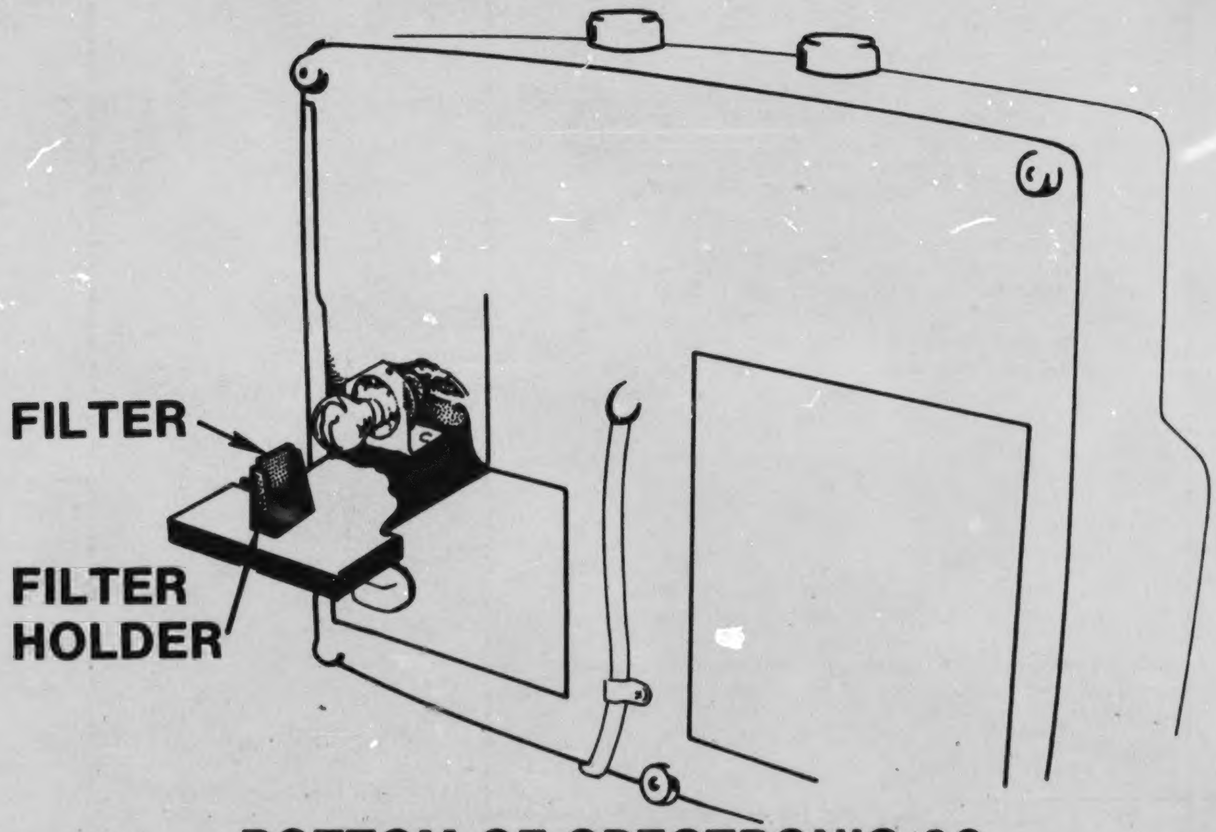
BOTTOM OF SPECTRONIC 20

FIGURE 4


PHOTOTUBE
FILTER HOLDER

BOTTOM OF SPECTRONIC 20

FIGURE 5


FILTER
FILTER HOLDER

BOTTOM OF SPECTRONIC 20

FIGURE 6

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Cell cleaning	1. Clean the Bausch & Lomb Spectronic 20 Spectrophotometer test tube cell.	1a. For the rest of this effluent monitoring procedure the abbreviation "Spec 20" will be used.	V.A.1.1 (p. 21)
2. Spec 20 cleaning	1. Clean the Spec 20.	1a. It should be free of dust, dirt, and spilled chemicals. 1b. The Spec 20 should be stored in an area where there is no danger that chemicals will be spilled on it. 1c. The plastic cover supplied with the Spec 20 should be covering the instrument whenever it is not in use.	
	2. If the power cord is plugged into a wall outlet, remove it.		
3. Phototube	1. Check whether the proper phototube is in place.	1a. See section C for instructions on changing the phototube and inserting the filter. 1b. On the wavelength scale, note that below about 625 nm, the numbers are in black, and that above 625 nm, the numbers are in red. 1c. If the wavelength to be used in the particular determination is in the black zone, the visible phototube (Bausch & Lomb Catalog number (33-29-71) should be used. 1d. If the wavelength to be used is in the red zone, the infra-red phototube (Bausch & Lomb Catalog number 33-29-72) and infra-red filter (Bausch & Lomb Catalog number 33-29-18) should be used.	
B. Spec 20			
1. Warm-up	1. Plug the power cord into a wall outlet.	1a. 115 V, A.C., 60 Hz	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Spec 20 (continued)	2. Turn the power switch/zero control knob (see figure 1) clockwise, until a click is heard.	2a. The instrument is now turned on. 2b. If there is a pilot light on the instrument, it will also be on. 2c. The sound of the cooling fan may also be heard.	V.B.1.2.2b (p. 22)
	3. Turn the power switch/zero control knob an additional one half clockwise turn.	3a. This will keep the needle from "pegging" during the warm-up period.	
	4. Wait ten minutes.	4a. This is the warm-up period. 4b. Ten minutes are generally specified in the manufacturer's manual. However, longer warm-up periods than those specified generally give better instrument stability. 4c. If the Spec 20 is old, a longer than 10 minute warm-up period may be required. Twenty to thirty minutes would be a suitable warm-up time.	
2. Operation	1. Assemble the standards and samples whose color intensities are to be measured.		
	2. Set the wavelength control to the desired setting.	2a. This setting will be specified in the procedure you are using to determine the particular parameter. 2b. Always approach the desired setting by turning the knob clockwise.	
	3. If the sample holder cover is open, close it.	3a. It should be closed unless a cell is being inserted or removed.	
	4. Turn the power switch/zero control knob until the needle reads infinite (symbol ∞) absorbance.	4a. Use the absorbance (lower) part of the scale. The other (upper) half of the scale is marked in transmittance.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Spec 20 (continued)		4b. The words absorbance and color intensity are related; i.e., if a solution has a low color intensity, it will also have a low absorbance.	
	5. Fill the cell with the blank.	5a. Also sometimes called the zero standard.	
	6. Empty the cell into the sink.		
	7. Fill the cell with blank.		
	8. Empty the cell into the sink.	8a. The cell has now been rinsed twice with solution.	
	9. Fill the cell with blank.	9a. Three fourths full. Estimate this volume.	
	10. Thoroughly wipe the outside of the cell with a tissue.	10a. So as to remove finger prints and any spilled solution.	
	11. Open the sample holder cover.		
	12. Slowly and gently slide the cell down into the sample holder as far as it will go.	12a. Do not force the cell down. 12b. The needle will move away from the infinite absorbance setting.	
	13. Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder (see figures 2 & 3).	13a. Be sure to rotate the cell slowly so that it is not scratched by the cell holder inside of the instrument.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Spec 20 (continued)	14. Close the sample holder cover.		
	15. Turn the light control knob until the needle reads zero absorbance.	15a. Use the absorbance scale for all of the readings.	
	16. Record an absorbance of zero and a concentration of zero for this solution.	16a. An example data sheet is on page 23.	
	17. Raise the sample holder cover.		
	18. Slowly remove the cell.	18a. No solution should be spilled on the inside of instrument.	
	19. Close the cover.	19a. The needle should return to the infinite absorbance setting. If it does not, reset it with the power switch/zero control knob. 19b. If it was necessary to reset the infinite absorbance reading, repeat steps 11 through 15.	
	20. Empty the contents of the cell into the sink.		
	21. Fill the cell with tap water.		
	22. Empty it into the sink.		
	23. Fill the cell with tap water.		
	24. Empty it into the sink.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Spec 20 (continued)	25. Fill the cell with distilled water.		
	26. Empty it into the sink.		
	27. Fill the cell with distilled water.		
	28. Empty it into the sink.		
	29. Fill the cell with the next solution whose color intensity (absorbance) is to be measured.	29a. In a set of standards, the absorbance of the lowest concentration standard is measured second, and so on, to the highest concentration standard.	
	30. Empty it into the sink.		
	31. Fill the cell with the same solution again.		
	32. Empty it into the sink.		
	33. Fill the cell three fourths full with the same solution.		
	34. Thoroughly wipe the outside of the cell with a tissue.	34a. So as to remove finger prints and any spilled solution.	
	35. Open the sample holder cover.		
	36. Slowly and gently slide the cell down into the sample holder as far as it will go.	36a. Do not force the cell down. 36b. The needle will move away from the infinite absorbance setting.	

EFFLUENT MONITORING PROCEDURE: Use of a Spectrophotometer

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Spec 20 (continued)	37. Slowly rotate the cell until the white vertical line on the cell is in line with ridge on the edge of the sample holder (see figures 2 & 3).		
	38. Close the sample holder cover.		
	39. Record the absorbance and concentration of this solution.	39a. While looking at the absorbance scale, note that in some parts of the scale, the third place to the right of the decimal will be an estimated number, while in other parts, the second place will be an estimated number. 39b. Absorbance values of greater than 0.7 are considered to be inaccurate. For this reason, about three sample dilutions are usually done so that at least one will give an absorbance of less than 0.7. If one of the standards happens to have an absorbance of greater than 0.7, it should not be used. 39c. If a great number of measurements are to be made at a particular time (e.g., a great number of phosphorus absorbancies are to be measured), steps 4 through 15 should be repeated every fifth measurement. 39d. Recall that step 4 was done with no cell in the instrument. 39e. This is an insurance aganist "drifting" of the setting.	
	40. Using each of the rest of the standards in sequence, and samples, repeat steps 17 through 39.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Spec 20 (continued)	41. Repeat steps 17 through 28.		
	42. Store the cell until it is again needed.		
	43. Turn the power switch/zero control knob slowly counterclockwise until a click is heard.	43a. If the instrument has a pilot light, it will go out. 43b. The Spec 20 is turned off.	
	44. If a plastic cover was supplied with the Spec 20, it should now be replaced.		
C. Phototube Changing	1. Turn the power switch/zero control knob slowly counter-clockwise until a click is heard.	1a. The instrument may already be turned off. 1b. If the instrument has a pilot light, it will go out. 1c. The Spec 20 is turned off.	
	2. Remove the power cord from the wall outlet.	2a. The power cord may already be removed from the wall outlet.	
	3. Tilt the Spec 20 away from you.	3a. The Spec 20 should be standing on its back. 3b. The bottom of the instrument is facing you. 3c. This position is somewhat unsteady. Be careful not to knock the instrument over.	
	4. Steady the instrument with one hand.		
	5. Loosen the thumbscrew with the other hand (see figure 4).		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Phototube Changing (continued)	6. Gently pull on the thumbscrew.	6a. So as to open the compartment door.	
	7. When removing the phototube to be replaced, grasp it with the finger tips (see figure 5).		
	8. Pull gently.	8a. A slight amount of wiggling may be needed.	
	9. Insert the other phototube, and, or, filter (see figure 6).		
	10. Close the compartment door.		
	11. Tighten the thumbscrew.		
	12. Return the Spec 20 to its normal position.		
	13. Continue with the EMP, Section B.		

EFFLUENT MONITORING PROCEDURE: Use of a Spectrophotometer

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required.	
	1. Pour 35 ml of distilled water in 250 ml beaker.	13th Standard Methods, p. 135, section 2.c.2
	2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dichromate, $Na_2Cr_2O_7$, to the water.	
	3. Swirl the beaker until the sodium dichromate has dissolved.	
	4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve.	
	5. Pour the solution into a 2 liter beaker.	
	6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker. Caution: Use eyeglasses and protective clothing.	
	7. Stir the mixture thoroughly.	
	8. Store it in a glass stoppered bottle.	
	9. The cleaning solution should be at a temperature of about 50°C when it is used.	
	10. It may therefore be necessary to warm the cleaning solution.	
	11. When using the warm cleaning solution, fill the piece of glassware with the solution.	
	12. Allow it to soak for 2-3 minutes (or longer).	
	13. Pour the cleaning solution back into the storage bottle.	
	14. Rinse the piece of glassware ten times with tap water.	
	15. The cleaning solution may be reused until it turns green.	
	16. It should then be discarded.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.1.2.2b.	There are two "versions" of the model 95 Spec 20. The regulated model has within it, electrical components which prevent fluctuations in current from affecting readings. The non-regulated model does not have this feature. Either "version" may, or may not, have a pilot light.	

EXAMPLE DATA SHEET

SPEC 20

A	C (mg/l)
0. _____	_____
0. _____	_____
0. _____	_____
0. _____	_____
0. _____	_____
0. _____	_____
0. _____	_____
0. _____	_____

A of sample = 0. _____

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

PREPARATION OF CALIBRATION GRAPHS

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Preparation of Calibration Graphs

This operational procedure was developed by:

NAME Charles R. Feldmann

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EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

M.S. - Chemistry

1-1/2 years Industrial Chemist

4 years additional Graduate School

4 years college Chemistry Instructor

1-1/2 years DHEW - Air Pollution Program, Chemist

4-1/2 years DI - EPA, Chemist-Instructor

EFFLUENT MONITORING PROCEDURE: Preparation of Calibration Graphs

1. Analysis Objectives:

The learner will prepare a calibration graph and will use it to determine the concentration of a chemical constituent in a sample of sewage effluent.

The word concentration means how much of the chemical constituent is present in a certain amount of sample; 1.0 milligram/liter is an example value of concentration.

2. Brief Description of Analysis:

In the field of water pollution analysis, calibration graphs are commonly used in two areas: absorbance and transmittance measurements. In the first case, energy is absorbed by some chemical constituent in a solution. In the second case, energy is transmitted by some chemical constituent in a solution. The amount of energy absorbed or transmitted can be related to the quantity of chemical constituent in a water sample by means of a calibration graph. Examples of absorbance measurements are colorimetric determinations, such as nitrate or phosphate using a spectrophotometer, and the determination of mercury or iron using atomic absorption. Examples of transmittance measurements are the determinations of sodium or potassium using flame photometry.

Two things must be done in order to prepare a calibration graph. A series of standards must be prepared. A standard is a solution which contains a known amount of the same chemical constituent which is being determined in the sample. Secondly, the absorbance or transmittance of these standards must be measured.

In order to actually determine how much of the chemical constituent is in the sample, the absorbance or transmittance of the sample must first be determined. The amount of chemical constituent is then read from the calibration graph.

For the sake of simplifying the instructions, absorbance values only will be used in the following procedure.

EFFLUENT MONITORING PROCEDURE: Preparation of Calibration Graphs

General Description of Equipment Used in the Process

A. Capital

None

B. Reusable

1. One ruler, 12 inches long
2. Pencil
3. Eraser

C. Consumable

1. Graph paper (one piece for each calibration graph). There are many kinds of graph paper. In ordinary water pollution analyses, a simple type of graph paper is used. Figure 1 is an example of the type of simple graph paper. The main feature of simple graph paper is that it is divided into a certain number of large squares of equal size. (For example, one inch might be the length of one side of the large squares). These large squares are subdivided into a certain number of smaller squares of equal size. (For example, a one inch square might be subdivided into one hundred small squares).

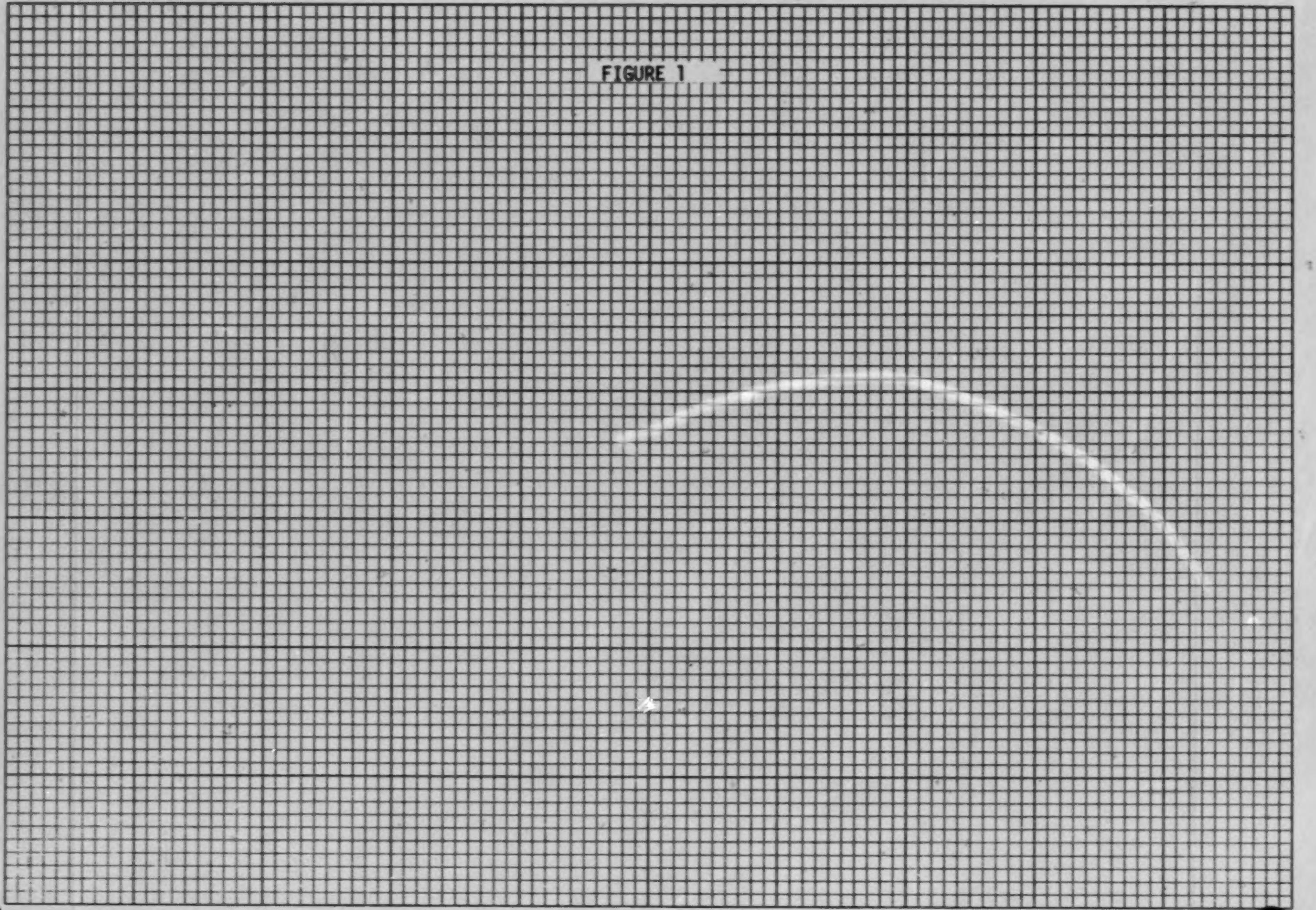


FIGURE 1

FIGURE 2

FIGURE 3

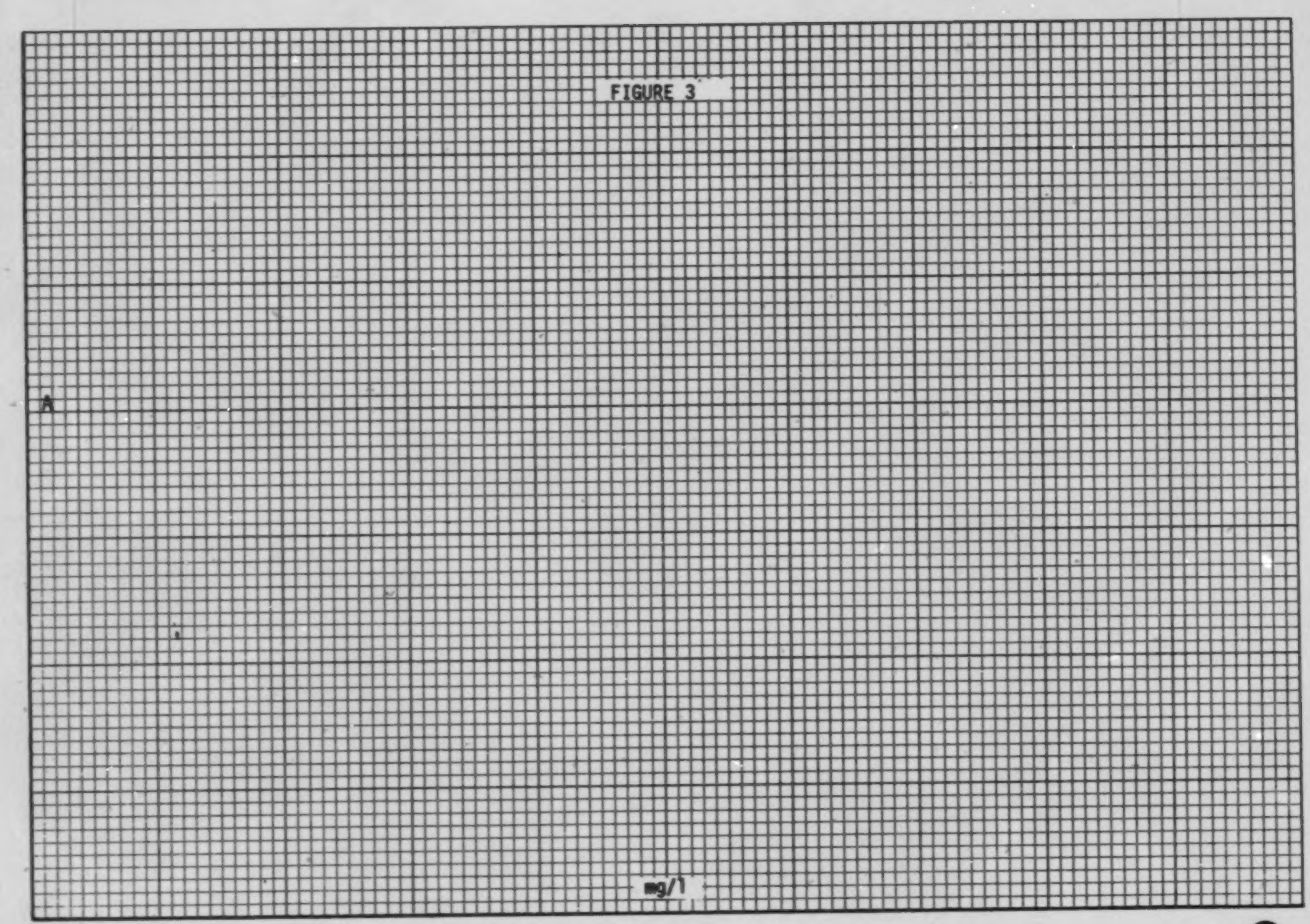
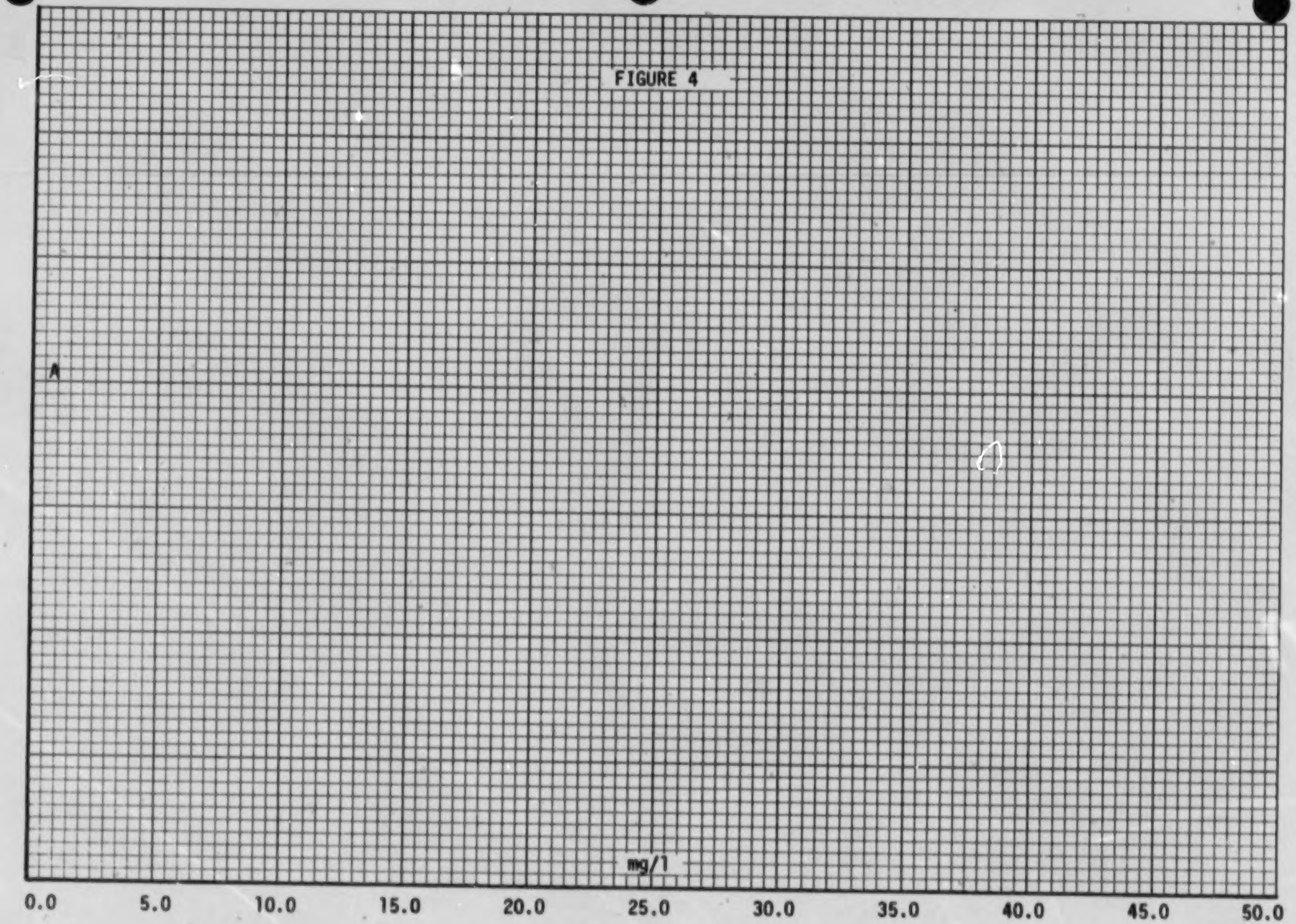


FIGURE 4



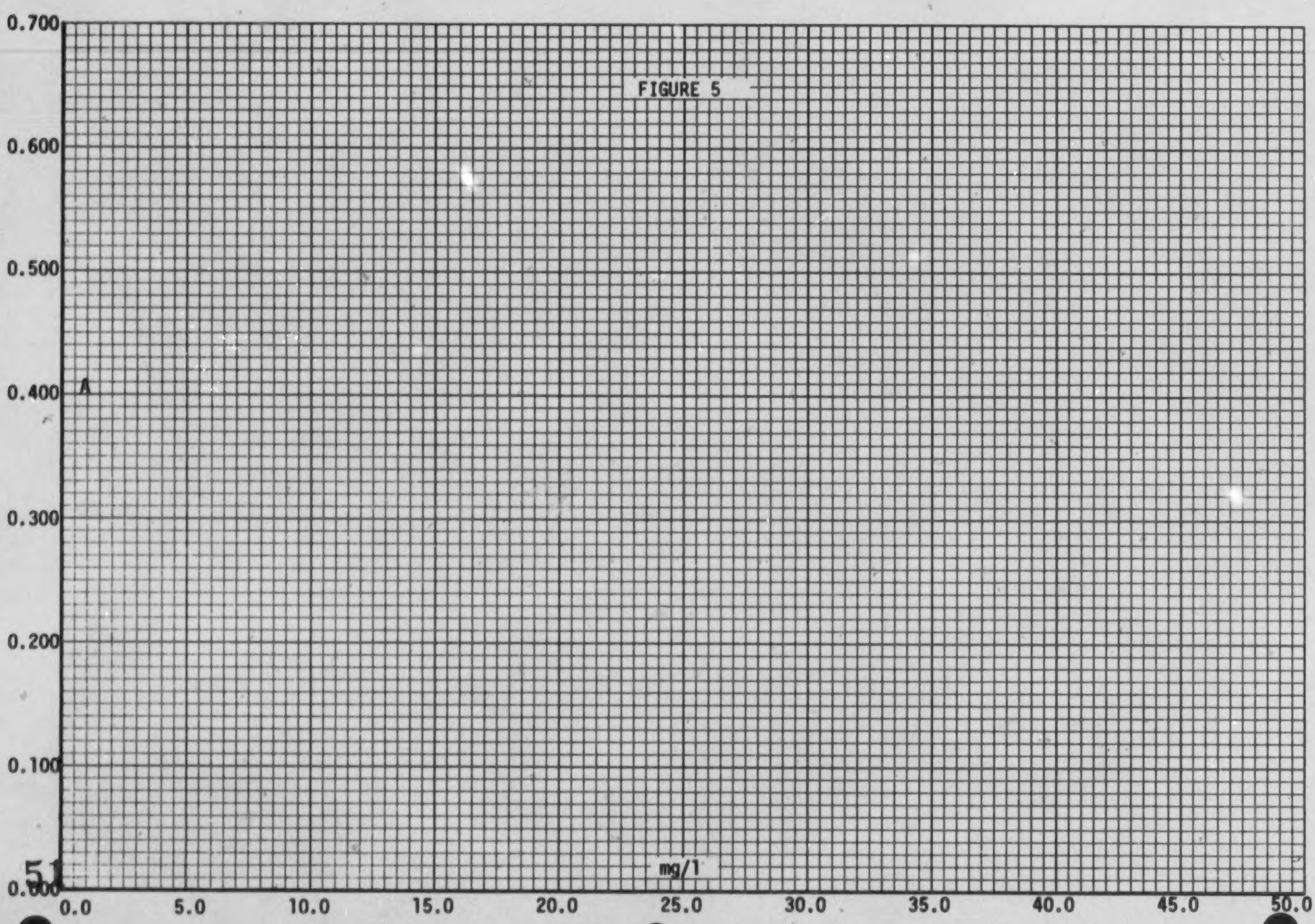


FIGURE 5

0.700
0.600
0.500
0.400
A
0.300
0.200
0.100
0.000
mg/1
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

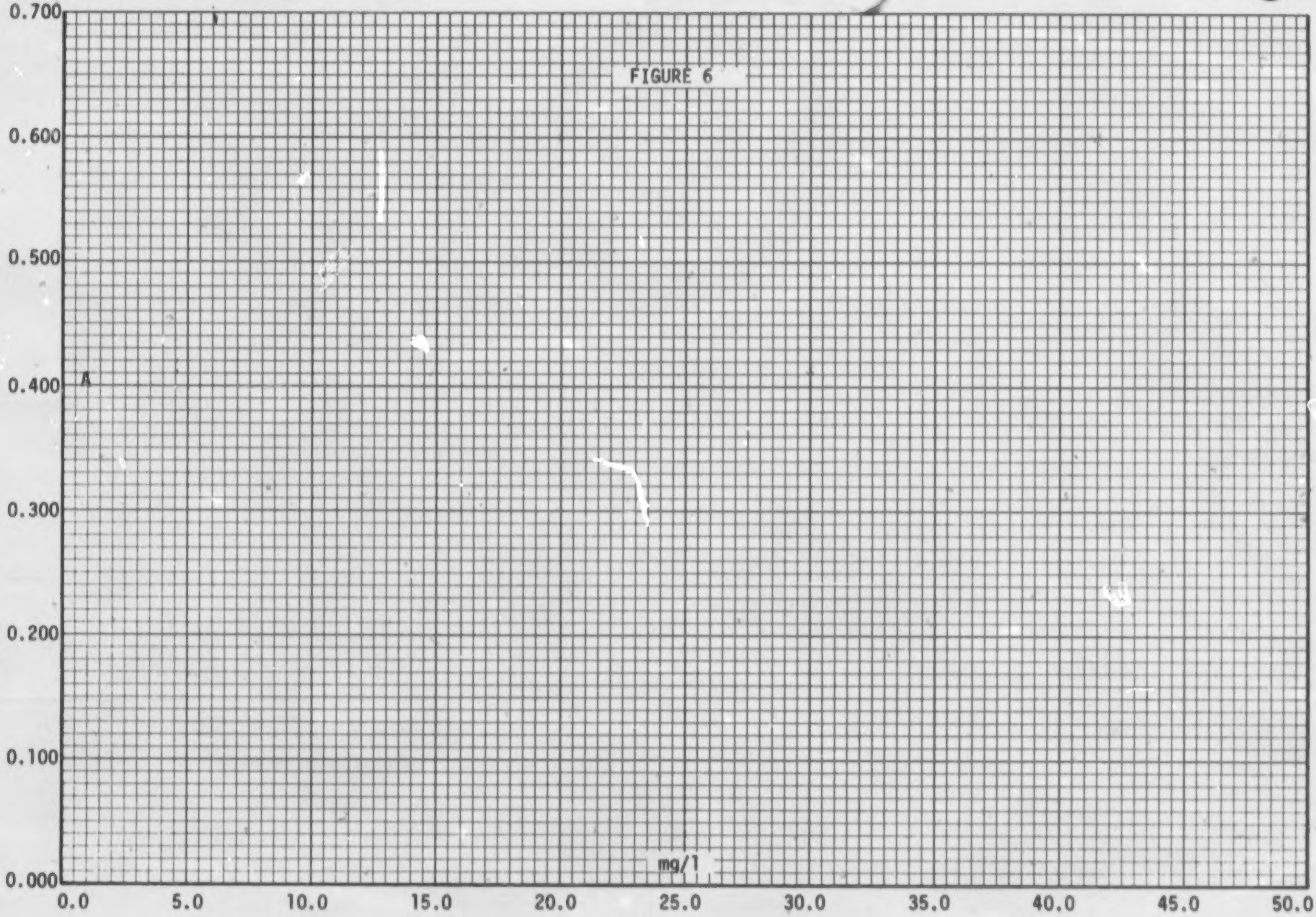


FIGURE 6

0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
A
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

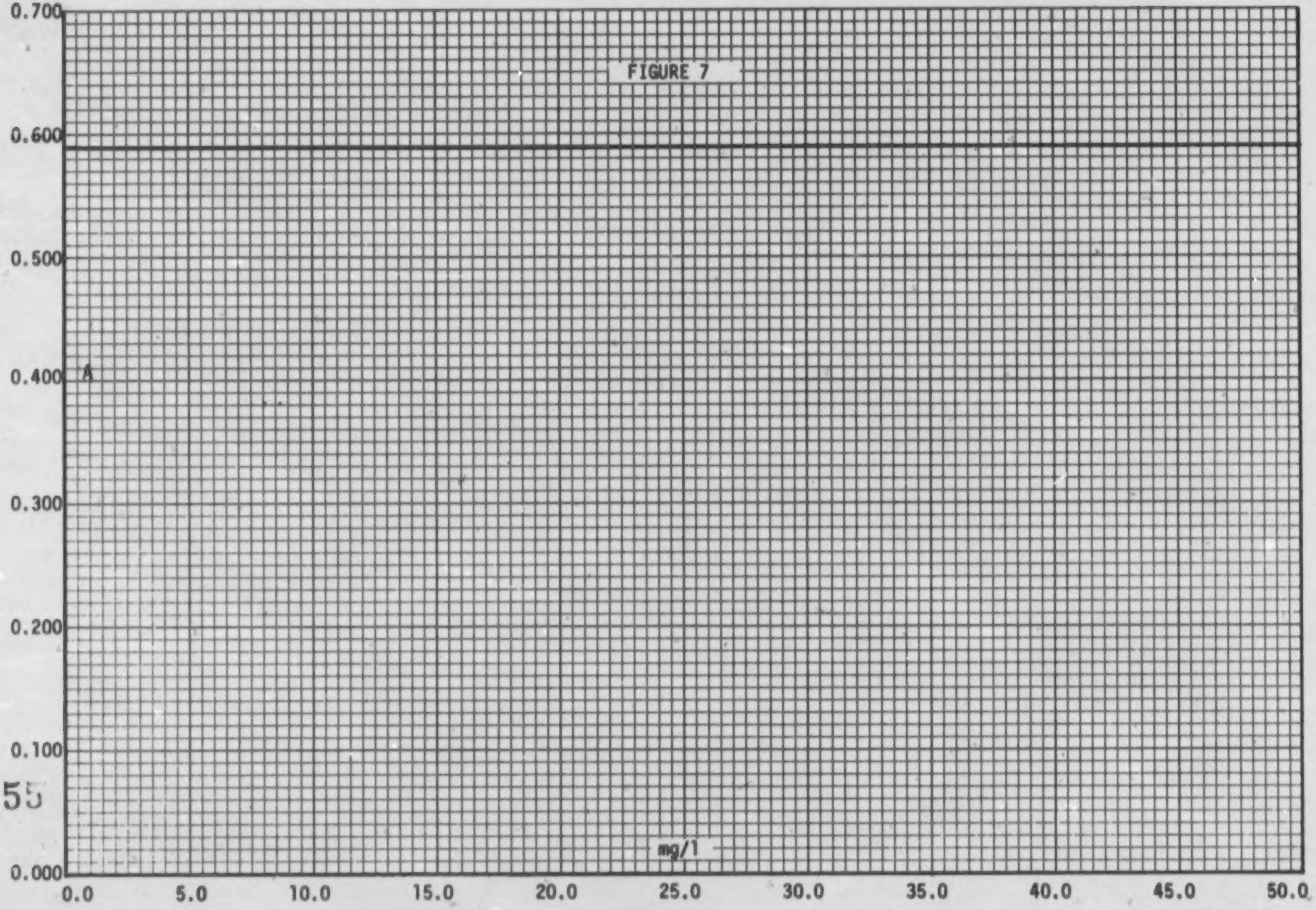

0.700
0.600
0.500
0.400
A
0.300
0.200
0.100
0.000
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 7

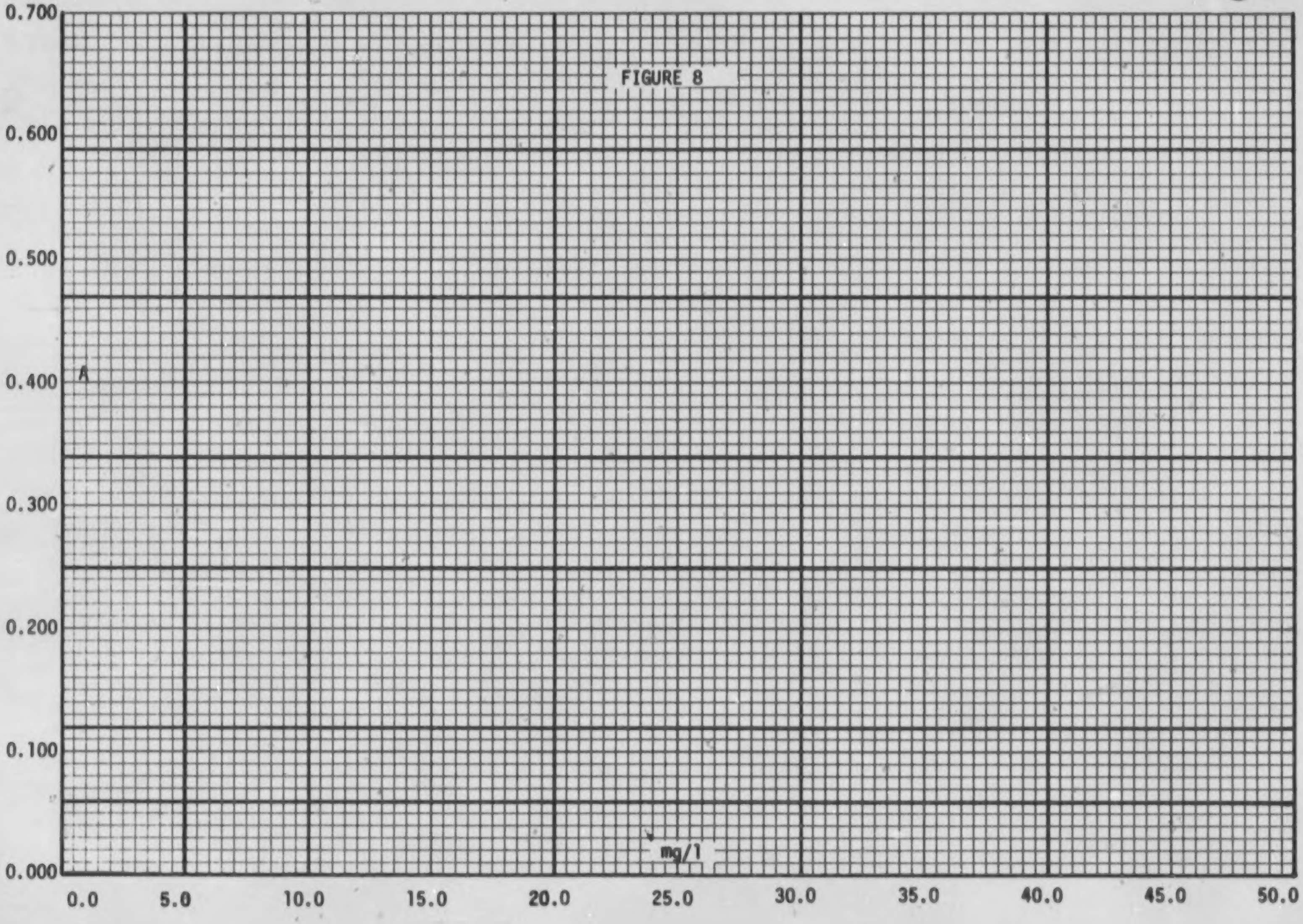


FIGURE 8

0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
A
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

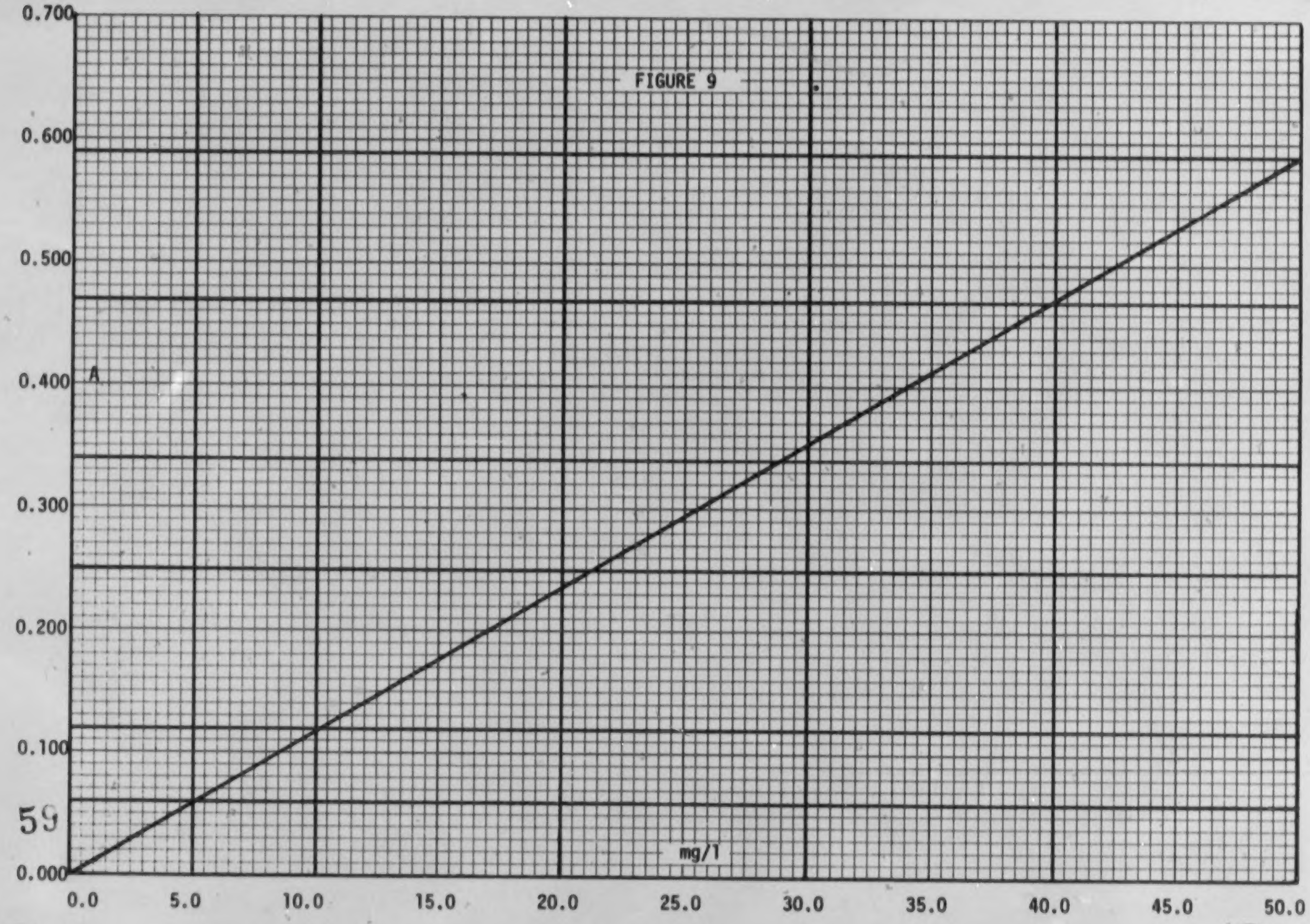


FIGURE 9
A
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
mg/l

59

60

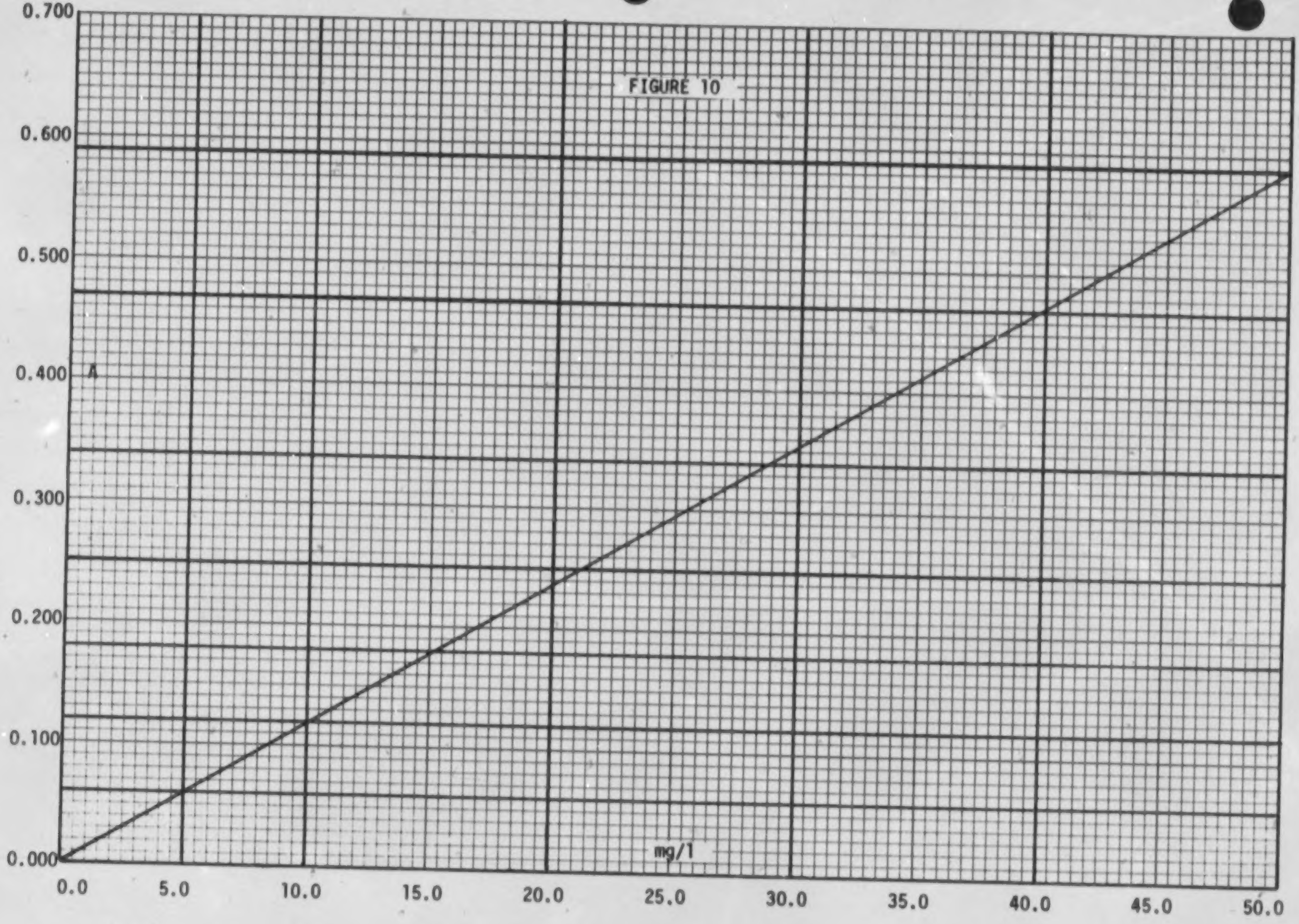


FIGURE 10

0.700
0.600
0.500
0.400
A
0.300
0.200
0.100
0.000
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

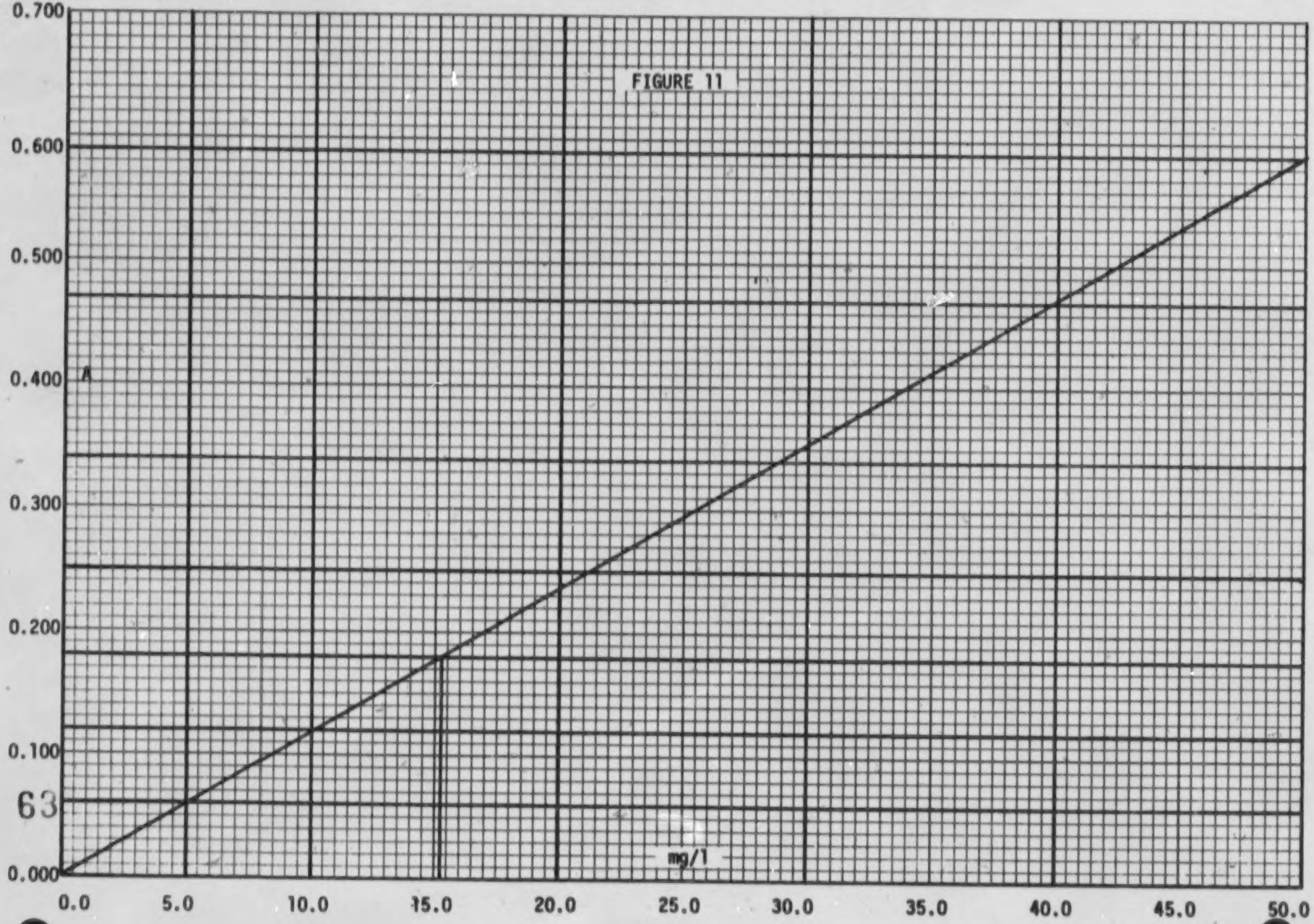


FIGURE 11
A
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

63

64

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Graph Paper			
1. General comments	1. Remove the page containing figure 1.		
	2. Lay it on a desk or any other place where it will be convenient for you to write on it.	2a. For the remainder of this procedure, you will actually use figure 1 and some example absorbance and concentration values to prepare a calibration graph. Additional figures are also included to demonstrate the instructions. 2b. You will have to furnish your own piece of graph paper when you want to prepare other calibration graphs.	
2. Labeling the graph paper	1. Draw two lines on figure 1 so that it looks like figure 2.	1a. Use a pencil, since you may have to do some erasing during the preparation of the calibration graph.	
	2. Label figure 1 so that it looks like figure 3.	2a. mg/l stands for milligrams per liter. It is an expression of concentration. If the amount of chemical constituent present in the sample is extremely small, the label µg/l (micrograms per liter) might be used. A stands for absorbance. 2b. The mg/l line is a horizontal line. It is called the X axis, or abscissa. The A line is called the Y axis, or ordinate.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Graph Paper (continued)	3. Examine the example absorbance and concentration values in the column at the right.	3a. mg/l — A 0.0 — 0.000 5.0 — 0.060 10.0 — 0.120 20.0 — 0.250 30.0 — 0.340 40.0 — 0.470 50.0 — 0.590 A of sample = 0.180 3b. It is data for a series of standards. 3c. Each pair of values (e.g. 5.0 and 0.060) represents a point on the graph. 3d. Later, you will complete the calibration graph by drawing a straight line through the seven points.	
	4. Note that the lowest mg/l value is 0.0 and the highest is 50.0.		
	5. Mark the mg/l axis on figure 1 so that it looks like figure 4.	5a. Note that the entire length of the mg/l axis was used. Always use as much of this line as is convenient. Do not, for example, use only one-half of the mg/l axis to mark off the values. 5b. Also note that each of the large squares is marked as a whole number of mg/l. 5c. Two of the smaller squares equal 1 mg/l.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Graph Paper (continued)	6. Note that the lowest A value is 0.000 and the highest is 0.590.	6a. It is generally not considered good practice to have A values greater than 0.6 or 0.7.	
	7. Mark the A axis on figure 1 so that it looks like figure 5.	7a. Note that the entire length of the A axis was used. Always use as much of this line as convenient. Do not, for example, use only one-half of the A axis to mark off the values.	
		7b. Also note that each of the large squares is marked as a whole number of A units.	
		7c. One of the smaller squares equals 0.01 A units.	
		7d. If transmittance measurements were being made, the Y axis or ordinate, would be marked T. T axes are always marked from 0 (bottom of axis) to 100 (top of axis).	
3. Drawing the calibration graph	1. On figure 1 draw a vertical line from the 50.0 mg/l point of the mg/l axis to the top of the graph.	1a. Figure 1 should now look like figure 6.	
	2. On figure 1 draw a horizontal line from the 0.590 point of the A axis to the right side of the graph.	2a. Figure 1 should now look like figure 7. 2b. The intersection of these two lines is the point represented by a concentration of 50.0 mg/l and an absorbance of 0.590.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Graph Paper (continued)	3. Using the same technique as in 1 and 2 above, locate the next five points on figure 1.	3a. The point located at 0.0 and 0.000 is at the intersection of the mg/l and A axes. 3b. Your graph should now look like figure 8. Some analyses may require more than five points.	
	4. Lay your ruler on figure 1.	4a. So one end of it lies at the 0.0 - 0.000 point, and at the 50.0 - 0.590 point.	
	5. Look along the edge of the ruler.	5a. The other five points (represented by the intersections of the horizontal and vertical lines do not all lie along the edge of the ruler.	
	6. Draw a line between the 0.0 - 0.000 and the 50.0 - 0.590 points.	6a. Note that some of the points lie slightly above the line, some lie slightly below the line, and some lie on the line. If one point is considerably off the line, some error in preparing the particular standard was probably made. 6b. This is the line of best fit for the seven points. Always draw the line of best fit when preparing calibration graphs. 6c. The calibration graph is now complete. 6d. Figure 1 should now look like figure 9. 6e. After you have prepared a few calibration graphs, you will find that you won't have to draw the horizontal and vertical lines to locate the points. You'll be able to move your pencil along the graph paper and put dots at the appropriate points. You'll then draw the line of best fit through them to the 0.0 - 0.000 point.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Determining the Concentration of the Chemical Constituent in the Sample.	1. Locate 0.180 on the A axis.	1a. This was the absorbance of the sample.	
	2. Draw a horizontal line to the right side of the paper.	2a. It should now look like figure 10.	
	3. Locate the intersection of this horizontal line and the sloping calibration graph.		
	4. From this intersection, draw a vertical line down to the bottom of the paper.	4a. It should now look like figure 11.	
	5. Note that the vertical line crosses the mg/l axis at 15.3.	5a. Recall that on the mg/l axis, 2 of the small squares equal 1 mg/l. 5b 15.3 mg/l is therefore the concentration of the chemical constituent being measured in the sample.	
C. Sample Dilution	1. If it was necessary to dilute the sample, the value read from the mg/l axis must be multiplied by a dilution factor.	1a. The dilution may have been necessary so that the A value for the sample would not be greater than the A value obtained for the highest concentration standard; 0.590 in this set of example data. 1b. The dilution factor is the ml of sample taken for dilution, divided into the ml to which it was diluted; e.g., if 10.0 ml of the original sample were diluted to 1000 ml (as in a volumetric flask) the dilution factor would be 1000/10, or 100. 1c. In some determinations, you may prepare more than one dilution of the sample. Look at the mg/l axis of figure 1 and assume that three dilutions of the sample gave values of 2.2, 24.0, and 48.0 mg/l, before correcting for the dilution factor. It is common practice to use the 24.0 value, since it lies nearest the middle of the calibration graph.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF TOTAL PHOSPHORUS (as P) OR OF ORTHOPHOSPHATE (as P), SINGLE REAGENT METHOD

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.PHOS.EMP.1a.3.76

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of Orthophosphate (as P), Single Reagent Method

This Operational Procedure was developed by:

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POSITION	Chemist-Instructor

EDUCATION AND TECHNICAL BACKGROUND

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2 years Industrial Laboratory Technician
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Wastewater Laboratory Analyst

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of Orthophosphate (as P), Single Reagent Method

1. Objective:

To determine orthophosphate, mg P/liter or total phosphorus, mg P/liter.

2. Description of Analysis:

Orthophosphate* in dilute solution will react with ammonium molybdate and antimony potassium tartrate to form a heteropoly acid. This acid is reduced to an intensely blue-colored complex, molybdenum blue, by ascorbic acid with the amount of blue produced being proportional to the amount of orthophosphate present.

In the procedure this is accomplished by the addition of a combined reagent to a 50 ml sample and a set of orthophosphate standards, followed by a wait for color development. A photometer or spectrophotometer is used to measure the absorbance of the samples and standards. The orthophosphate concentrations of samples are read directly from a graph prepared by plotting the absorbance values of the standards against their concentration.

This analytical procedure utilizes reactions that are specific for the orthophosphate ion. In order to obtain the total phosphorus concentrations of samples, all non-orthophosphate phosphorus forms must be converted to the orthophosphate ion. In the procedure this is accomplished by digesting samples with ammonium persulfate and sulfuric acid. This step does not affect the original orthophosphate content of the sample, but ensures conversion of all other forms of phosphorus to orthophosphate. Direct orthophosphate colorimetry may then be performed on the sample as described in the preceding paragraph, and the results obtained reported as total phosphorus, mg P/liter.

*The orthophosphate ion, $(PO_4)^{\equiv}$ ion, is the smallest and simplest of the phosphorus-oxygen radicals. It consists of four oxygen atoms tetrahedrally arranged around and bonded to a central phosphorus atom. The more complex and commercially important phosphates, the poly or multiphosphates (P_2O_7, P_3O_{10}, etc.), are typically formed by linking orthophosphate units. The term "phosphate" is a general one and may apply to any one of hundreds of compounds. The $(PO_4)^{\equiv}$ ion is distinguished by the prefix "ortho" and is correctly called the orthophosphate ion.

3. Applicability of this Procedure:

a. Range of Concentration:

0.01 to 1.00 mg P/liter
(The range may be extended for samples by dilution.)

b. Pretreatment of Samples:

This procedure includes the persulfate digestion for the total Phosphorus determination as specified in the Federal Register Guidelines. These Guidelines do not specify any pretreatment for the orthophosphate determination.

c. Treatment of Interferences in Samples:

This procedure includes directions for removal of turbidity or suspended solids from samples for the orthophosphate determination. It also includes the modification to prevent adsorption of phosphorus on metal precipitates in samples for the total phosphorus determination as publicized in the "Changes and Errata. . ." for the Source of Procedure*. For either determination it includes the treatment for samples which have been preserved with mercury chloride. Arsenate is the one additional interference listed in the Source of Procedure*. No remedy for its presence is currently available, but one should be aware that arsenate also responds to this analysis and can contribute to erroneously high phosphorus values.

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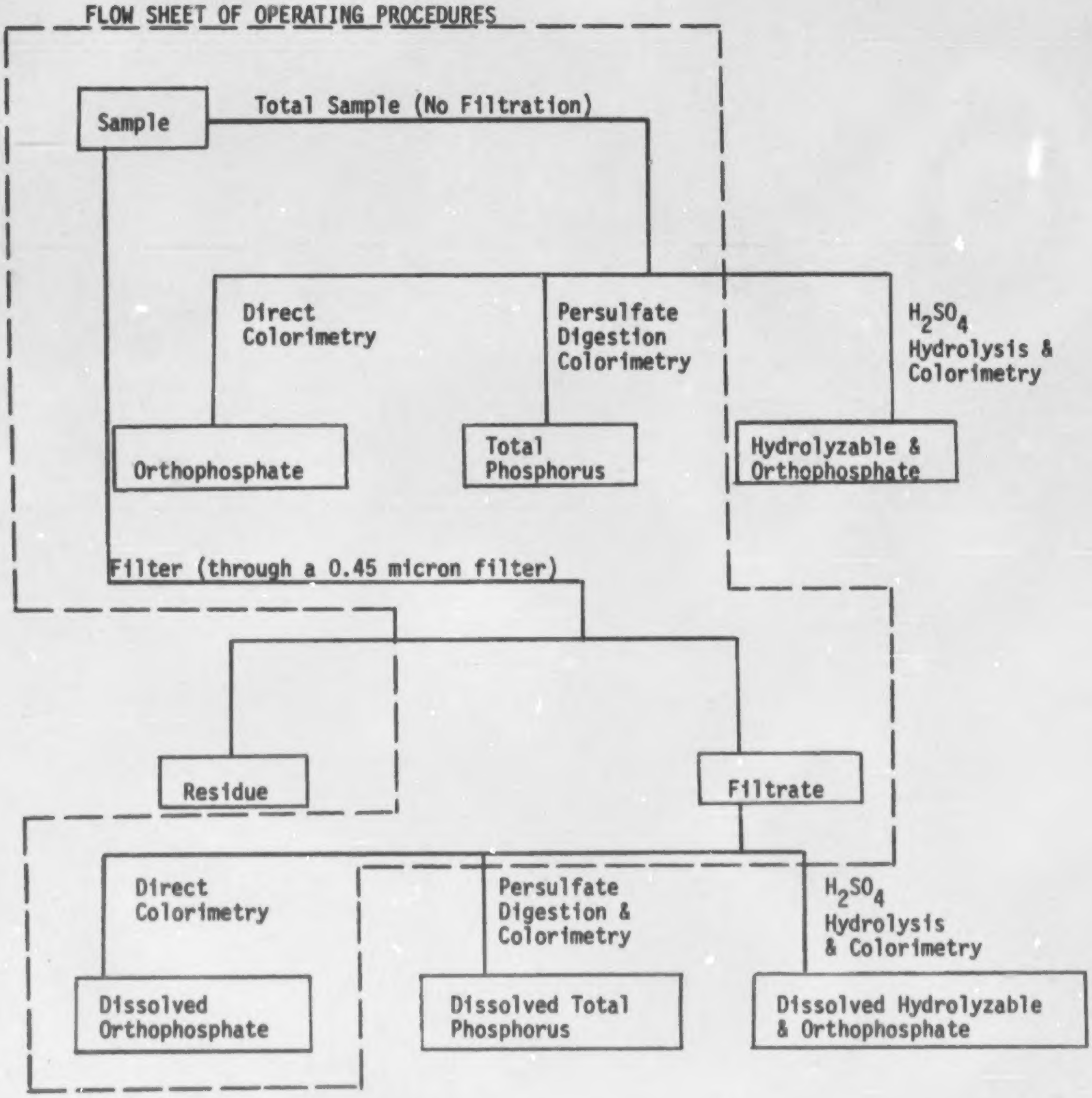
*Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, Ohio, page 249.

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of Orthophosphate (as P), Single Reagent Method


FLOW SHEET OF OPERATING PROCEDURES
Sample
Total Sample (No Filtration)
Direct Colorimetry
Persulfate Digestion Colorimetry
H_2SO_4 Hydrolysis & Colorimetry
Orthophosphate
Total Phosphorus
Hydrolyzable & Orthophosphate
Filter (through a 0.45 micron filter)
Residue
Filtrate
Direct Colorimetry
Persulfate Digestion & Colorimetry
H_2SO_4 Hydrolysis & Colorimetry
Dissolved Orthophosphate
Dissolved Total Phosphorus
Dissolved Hydrolyzable & Orthophosphate

This EMP includes only the material within the dotted line area.

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of
Orthophosphate (as P), Single Reagent Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Balance, triple-beam, capable of 0.1 gram sensitivity
2. Balance, analytical, capable of weighing to 0.1 mg under a 200 g load
3. Desiccator
4. Hot plate or plates, capable of holding a minimum of ten 125 ml Erlenmeyer flasks or an
   autoclave, capable of 121°C (15-20 psi), with capacity for a minimum of ten 125 ml Erlenmeyer flasks
5. Oven, drying, for use at 105°C
6. pH meter, electric, equipped with single combination electrode, capable of ± 0.1 pH unit sensitivity
7. Refrigerator, capable of maintaining a 4°C temperature
8. B and L Spectronic 20 (or equivalent) spectrophotometer equipped with accessory infrared phototube and filter capable of operation at 650 or 880 nm or a
   filter photometer, equipped with red filter or a
   spectrophotometer, uv-visible, capable of operation at 650 nm or 880 nm
9. Vacuum source or pump drawing 15 inches mercury

B. Reusable Supplies:

1. One apron, laboratory
2. One pound glass beads, 5 mm diameter, for smoothing boiling action
3. One beaker, 250 ml
4. One beaker, 1000 ml
5. One beaker, 1500 ml
6. Two 100 ml bottles, glass or plastic with caps
7. One shallow, open mouthed bottle
8. Three 500 ml bottles, plastic with caps
9. One 500 ml bottle, dark glass with stopper
10. Two 1000 ml bottles, glass with stoppers or caps
11. Two 1000 ml bottles, plastic with caps
12. One 2000 ml bottle, glass with cap
13. One bulb, rubber for pipetting
14. One 25 ml cylinder, graduated
15. One 100 ml cylinder, graduated
16. One 500 ml cylinder, graduated
17. One 1000 ml cylinder, graduated
18. One evaporating dish, porcelain, 100 ml, to contain ammonium persulfate
19. One evaporating dish, porcelain, 35 ml to dry potassium dihydrogen phosphate
20. XXX membrane filter assembly with funnel in a #7 stopper to fit the mouth of a 500 ml suction flask. One as minimum, faster with nine plus one for each sample.
21. XXX 500 ml suction flask with side arm--one for each filter assembly
22. XXX 50 ml flasks, volumetric with stoppers, nine + one for each sample
23. One 500 ml flask, volumetric with stopper

B. Reusable Supplies (Cont'd.):

24. One 1000 ml flask, volumetric with stopper
25. XXX 125 ml flasks, Erlenmeyer, graduated, nine plus one for each sample
26. Two funnels: 1 glass, powder and 1 to fit 50 ml volumetric flask
27. One pair rubber gloves for washing glassware with acid solution
28. One pair goggles or safety glasses
29. XXX hose lengths for connecting suction flasks to vacuum sources
30. One 1 ml pipet, graduated in 0.1 ml
31. One 1 ml pipet, volumetric
32. One 3 ml pipet, volumetric
33. One 5 ml pipet, volumetric
34. One 10 ml pipet, volumetric
35. One 20 ml pipet, volumetric
36. One 30 ml pipet, volumetric
37. One 50 ml pipet, volumetric
38. Two 10 ml pipets, graduated (Mohr)
39. One pneumatic trough or small pan for cold-water bath
40. One respirator if a hood is not available
41. One spatula
42. One 0.4 g measuring spoon, Hach or equivalent (optional)
43. One 8 inch stirring rod, glass
44. One pair tongs
45. One wash bottle, squeeze type

C. Consumable Supplies:

NOTE: All reagents must be of high purity, such as "A.C.S.," "reagent grade," or "analyzed"

1. Water, distilled (as needed)
2. Hydrochloric acid (HCl), concentrated, 1 pint minimum
3. Sulfuric acid (H_2SO_4), concentrated, 1 pint minimum
4. Antimony potassium tartrate $[K(SbO)C_4H_4O_6 \cdot 1/2\ H_2O]$ (recommend purchase of 1 lb. units)
5. Ammonium molybdate $[(NH_4)_6\ Mo_7O_{24} \cdot 4H_2O]$ (recommend purchase of 1 lb. units)
6. Ascorbic acid (recommend purchase of 5-ounce units)
7. Ammonium persulfate $[(NH_4)_2S_2O_8]$ (recommend purchase of 1 lb. units)
8. Potassium dihydrogen phosphate (KH_2PO_4) (recommend purchase of 1 lb. units)
9. Sodium hydroxide (NaOH) (recommend purchase of 1 lb. units)
10. *Mercuric chloride ($HgCl_2$)
11. *Sodium chloride (NaCl)
12. Boats, weighing, plastic disposable

*Only needed if samples must be preserved (i.e. if analysis cannot be performed on the same day that the sample was collected).

13. Filters, 0.45 micron pore size membrane, phosphorus-free, Gelman GA 6 or equivalent
14. Notebook, bound laboratory, for permanently recording data
15. Paper, graph: 8 1/2 inch by 11 inch dimestore school supply is suitable. Recommend graph paper have seven major divisions along 8 1/2 inch side and 10 major divisions along 11 inch side.
16. Tape, labeling, one roll (masking tape is suitable)
17. Tissue, lint-free, for wiping colorimeter tubes or cuvettes

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
TOTAL PHOSPHORUS (as P) OR OF ORTHOPHOSPHATE (as P), SINGLE REAGENT METHOD			I (p. 39)
A. Glassware Preparation	1. Assemble all necessary equipment.	1a. See pages 7-9 for list of necessary equipment.	
	2. Heat 500 ml 1:1 HCl.	2a. In a 1000 ml beaker. 2b. Use a hot plate or bunsen burner. 2c. For directions on making 1:1 HCl, See B, "Reagent Preparation." 2d. CAUTION: Use extreme precautions with hot 1:1 HCl acid. This solution will cause severe burns. Wear gloves, apron, goggles, etc., while handling. Vapor from hot acid is extremely irritating to eyes and throat. Use a hood or wear a respirator while using.	
	3. Rinse all glassware to be used in procedure.	3a. Use hot 1:1 HCl.	
	4. Discard all 1:1 HCl used in rinsing glassware.	4a. CAUTION: 1:1 HCl carelessly poured down drains will quickly eat out traps.	
	5. Flush away discarded 1:1 HCl.	5a. Use plenty of tap water.	
	6. Rinse the glassware with tap water.	6a. Fill and empty two times.	
	7. Rinse the glassware with distilled water.	7a. Use several portions of distilled water.	
	8. Rinse the glassware with combined reagent.	8a. One time. 8b. For directions on making combined reagent, see B, Reagent Preparation.	

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Glassware Preparation (continued)		8c. The combined reagent will turn blue on contact with orthophosphates. 8d. The purpose of this cleaning procedure is to remove all phosphates. Appearance of blue color on combined reagent-rinsed glassware is indicative of failure of first cleaning or else phosphate contamination in the distilled water.	
	9. Check all combined reagent-rinsed glassware.	9a. After 10 minutes contact with the combined reagent. 9b. Look for blue color. 9c. For any glassware showing blue color, repeat steps 3 through 9 of this Operating Procedure. 9d. For any glassware not responding with blue color in the combined reagent, proceed to step 10. 9e. If any glassware shows blue color after second cleaning, have distilled water checked for phosphates.	
	10. Rinse the glassware with distilled water.	10a. Use generous amounts.	
B. Reagent Preparation			
1. 1:1 hydrochloric acid	1. Measure out 1000 ml distilled water.	1a. Use a 1000 ml (1 liter) graduated cylinder.	
	2. Pour the water into a clean glass bottle.	2a. Bottle must have a capacity greater than 2 liters.	
	3. Measure out 1000 ml concentrated hydrochloric acid (HCl).	3a. Use a 1000 ml graduated cylinder. 3b. CAUTION: Hydrochloric acid causes severe burns. Vapor is extremely irritating. Use care when handling.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	4. Slowly, pour the 1000 ml of concentrated HCl into the bottle.	4a. Avoid spattering the acid by holding the bottle at an angle so that the acid runs down the side.	
	5. Gently swirl the bottle to mix the contents.		
	6. Label the bottle "1:1 Hydrochloric Acid."	6a. The date and initials of preparer should always be included on the label of any reagent container.	
2. 10 N sodium hydroxide	1. Prepare a shallow cold-water bath.	1a. In a small pan or pneumatic trough.	
	2. Weigh out about 40 grams sodium hydroxide (NaOH) pellets as rapidly as possible.	2a. In a tared weighing boat on a triple beam balance. 2b. Sodium hydroxide rapidly picks up moisture.	
	3. Transfer the pellets to a 250 ml beaker.		
	4. Measure out 100 ml of distilled water.	4a. Use a 100 ml graduated cylinder.	
	5. Place the 250 ml beaker in the prepared cold-water bath.		
	6. Slowly pour the 100 ml of distilled water into the reagent container.		
	7. Gently stir the beaker's contents in the cold-water bath.	7a. Use a glass stirring rod. 7b. To mix the contents and cool the solution to room temperature.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	8. When solution is cool, remove the beaker from the water bath and pour the solution into a reagent container.	8a. Reagent container must be 100 ml capacity or greater. 8b. NOTE: Plastic storage containers are preferable for sodium hydroxide (NaOH) solutions as they will etch glass over a period of time, resulting in a loss of strength of the solution.	
	9. Label the reagent bottle "10 N Sodium Hydroxide."	9a. Solution is indefinitely stable if container is kept tightly capped when not in use to prevent admittance to atmospheric carbon dioxide (CO_2) gas.	
3. 0.1 N sodium hydroxide	1. Measure out 10 ml of the 10 N sodium hydroxide.	1a. Use a 25 ml graduated cylinder. 1b. This is reagent #2, above.	
	2. Pour the 10 ml of 10 N sodium hydroxide into a reagent container.	2a. The reagent container should be 1 liter capacity or greater. 2b. A plastic reagent container is preferred, as sodium hydroxide etches glass.	
	3. Measure out 900 ml of distilled water.	3a. Use a 1000 ml graduated cylinder.	
	4. Slowly pour the distilled water into the reagent container with the 10 ml 10 N sodium hydroxide.		
	5. Swirl the container.	5a. To thoroughly mix the contents.	
	6. Label this reagent container "0.1 N Sodium Hydroxide."	6a. This reagent will be used solely for adjusting the pH of samples and standards.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
4. Strong acid solution, 11 N sulfuric acid	1. Measure out 600 ml distilled water.	1a. Use a 1000 ml graduated cylinder.	
	2. Pour the distilled water into a 1500 ml beaker.		
	3. Measure out 310 ml concentrated sulfuric acid (H_2SO_4).	3a. Use a 500 ml graduated cylinder. 3b. CAUTION: Contact with concentrated sulfuric acid causes severe burns.	
	4. Place the 1500 ml beaker in a cold-water bath.	4a. In a small pan or pneumatic trough.	
	5. Very slowly pour the 310 ml concentrated sulfuric acid into the 1500 ml beaker.	5a. Hold the beaker at an angle, so the acid runs down the side of the container. 5b. CAUTION: If the acid is added too quickly, the water will boil and spatter the sulfuric acid.	
	6. Gently stir the contents of the beaker in the cold-water bath.	6a. Use a glass stirring rod. 6b. To mix the contents. 6c. Let the reagent container stand in the cold-water bath while the solution cools to room temperature.	
	7. Measure out 90 ml of distilled water.	7a. Use a 100 ml graduated cylinder.	
	8. Slowly pour the 90 ml of distilled water into the 1500 ml beaker.		
	9. Gently stir the contents of the 1500 ml beaker.	9a. Use a glass stirring rod. 9b. To thoroughly mix the contents.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	10. When solution is cool, remove the beaker from the water bath and pour the solution into a reagent container.	10a. Reagent container may be either glass or plastic. 10b. It must be 1 liter capacity or greater.	
	11. Label the reagent container "Strong Acid Solution."	11a. Solution is indefinitely stable.	
5. 1.1 N Sulfuric acid	1. Measure out 900 ml of distilled water.	1a. Use a 1000 ml graduated cylinder.	
	2. Pour the distilled water into a reagent container.	2a. The reagent container should be glass, 1 liter capacity or greater.	
	3. Measure out 100 ml of 11 N sulfuric acid.	3a. Use a 100 ml graduated cylinder. 3b. This is reagent #4, above.	
	4. Slowly pour the 100 ml of 11 N sulfuric acid into the reagent container with the distilled water.		
	5. Swirl the reagent container.	5a. To thoroughly mix the contents.	
	6. Label this reagent container "1.1 N Sulfuric Acid."	6a. This reagent will be used solely for adjusting the pH of samples and standards.	
6. 5 N sulfuric acid	1. Measure about 400 ml distilled water.	1a. Use a 500 ml graduated cylinder.	
	2. Pour the distilled water into a 500 ml volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	3. Measure out 70 ml concentrated sulfuric acid (H_2SO_4).	3a. Use a 100 ml graduated cylinder. 3b. CAUTION: Contact with sulfuric acid causes severe burns.	
	4. Place the volumetric flask in the cold-water bath.	4a. In a small pan or pneumatic trough.	
	5. Slowly pour the 70 ml concentrated sulfuric acid into the flask.	5a. Hold the flask at an angle, so the acid runs down the side of the flask. 5b. CAUTION: If the acid is added too quickly, the water will boil and spatter the sulfuric acid.	
	6. Gently swirl the flask in the cold-water bath.	6a. To mix the contents and cool the solution to room temperature.	
	7. When solution is cooled to room temperature, add distilled water to bring solution to 500 ml volume.		
	8. Transfer the solution to a 500 ml plastic storage container.	8a. Container should be labeled "5 N Sulfuric Acid." 8b. Prepare this solution weekly.	
7. Antimony potassium tartrate solution	1. Weigh out exactly 1.3715 grams of antimony potassium tartrate [$K(SbO)C_4H_4O_6 \cdot 1/2\ H_2O$].	1a. In a weighing boat. 1b. Use an analytical balance. 1c. Observe all handling precautions given on the reagent bottle label.	
	2. Quantitatively (that is, completely) transfer the 1.3715 grams of antimony potassium tartrate to a 500 ml volumetric flask.	2a. Funnel the chemical into the flask, using a distilled water squirt bottle to wash all traces of the chemical from the weighing boat and powder funnel into the flask. 2b. CAUTION: Use minimum amount of distilled water necessary.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	3. Measure out 400 ml of distilled water.	3a. Use a 500 ml graduated cylinder.	
	4. Pour the 400 ml of distilled water into the flask.		
	5. Swirl the flask gently.	5a. Until the chemical has dissolved.	
	6. Dilute the contents of the flask to 500 ml.		
	7. Transfer the solution to a clean storage bottle.	7a. Bottle must be 500 ml capacity or greater. 7b. Bottle must be dark and glass-stoppered.	
	8. Label the storage bottle "Antimony Potassium Tartrate Solution."	8a. Store this solution in the dark at 4°C.	
8. Ammonium molybdate solution	1. Weigh out 20 grams ammonium molybdate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$.	1a. In a weighing boat. 1b. Use a triple-beam (0.1 g sensitivity) balance.	
	2. Measure out 500 ml of distilled water.	2a. Use a 500 ml graduated cylinder.	
	3. Transfer the 20 grams of ammonium molybdate to a plastic storage bottle.	3a. Use a powder funnel and distilled water squirt bottle to wash all traces of the chemical from the weighing boat and powder funnel into the storage bottle. 3b. Bottle must be 500 ml capacity or greater. 3c. Use a minimum of distilled water.	
	4. Rinse any remaining chemical from the weighing boat into the plastic storage bottle.	4a. Use part of the 500 ml distilled water measured out in step 2.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	5. Pour the remaining distilled water into the plastic storage bottle.		
	6. Gently swirl the plastic bottle.	6a. To dissolve the ammonium molybdate.	
	7. Label the bottle "Ammonium Molybdate Solution."	7a. Store this solution at 4°C. 7b. Prepare this solution weekly.	
9. 0.1 M ascorbic acid	1. Weigh out 1.76 grams of ascorbic acid.	1a. In a weighing boat. 1b. Use an analytical balance.	
	2. Measure out 100 ml distilled water.	2a. Use a 100 ml graduated cylinder.	
	3. Transfer the 1.76 g ascorbic acid to a storage bottle.	3a. Storage bottle must be 100 ml capacity or greater. 3b. Storage bottle may be either plastic or glass. 3c. Use part of the 100 ml of distilled water measured out in step 2 to rinse any remaining traces of ascorbic acid from the weighing boat into the storage bottle.	
	4. Pour the remaining distilled water into the storage bottle.		
	5. Gently swirl the storage bottle.	5a. To dissolve the ascorbic acid.	
	6. Label the bottle "Ascorbic Acid Solution."	6a. Store the solution at 4°C. 6b. Prepare this solution weekly.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
10. Combined reagent (combination of reagents 6, 7, 8, and 9 above)	1. Bring reagents 6, 7, 8, and 9 to room temperature before doing the following steps.	1a. It is critical that all solutions used in the makeup of this combined reagent be at room temperature before mixing, and that they be mixed in the order given.	
	2. Measure 50 ml 5 N sulfuric acid into a storage container.	2a. Use a 100 ml graduated cylinder. 2b. Solution must be at room temperature. 2c. Storage container may be either glass or plastic. 2d. Storage container must be 100 ml capacity or greater.	
	3. Pipet 5 ml antimony potassium tartrate solution into the storage bottle.	3a. Use a 5 ml volumetric pipet and a rubber bulb. 3b. Solution must be at room temperature before addition.	
	4. Gently swirl the storage bottle.	4a. To thoroughly mix the contents. 4b. If any turbidity (cloudiness) is observed, shake the bottle and allow it to stand for a few minutes until the turbidity disappears before proceeding to step 5.	
	5. Measure 15 ml ammonium molybdate solution into the storage bottle.	5a. Use a 25 ml graduated cylinder. 5b. Solution must be at room temperature before addition.	
	6. Gently swirl the bottle.	6a. To thoroughly mix the contents. 6b. If any turbidity (cloudiness) is observed, shake the bottle and allow to stand for a few minutes until the turbidity disappears before proceeding to step 7.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	7. Measure 30 ml ascorbic acid solution into the storage bottle.	7a. Use a 100 ml graduated cylinder. 7b. Solution must be at room temperature before addition.	
	8. Gently swirl the bottle.	8a. To thoroughly mix the contents. 8b. If any turbidity (cloudiness) is observed, shake the bottle and allow the combined reagent to stand for a few minutes until the turbidity disappears before using the combined reagent.	
	9. Label the storage bottle "Combined Reagent."	9a. The combined reagent is extremely unstable and must be prepared fresh before each use. 9b. This 100 ml of combined reagent is sufficient for 12 determinations. If large numbers of samples are to be run simultaneously, larger quantities of the combined reagent may be prepared by using the same reagent proportions.	
11. Ammonium persulfate	1. Transfer about 50 grams of ammonium persulfate $(NH_4)_2S_2O_8$ into a container.	1a. Use a spatula. 1b. Put into any open-mouthed, shallow container convenient to scoop or weigh from. 1c. CAUTION: This is a vigorous oxidizing agent.	
	2. Label the container "Ammonium Persulfate."	2a. Store in a desiccator. 2b. Prevent contact with any combustible material.	V.B.11.2a (p. 41)
12. Stock phosphorus solution	1. Preheat an oven to 105°C.	1a. An oven used for drying suspended solids crucibles or filters is suitable.	
	2. Transfer a few grams of potassium dihydrogen phosphate (KH_2PO_4) to a suitable container.	2a. Use a spatula. 2b. NOTE: Any shallow, open container is suitable as long as it can withstand 105°C heat. A small porcelain evaporating dish is handy for the purpose.	

EFFLUENT MONITORING PROCEDURE: Determination of Total PHosphorus (as P) or of Orthophosphate (as P), Single Reagent Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	3. Transfer the container of potassium dihydrogen phosphate to the preheated oven.	3a. Use tongs. 3b. This will drive off atmospheric moisture that the chemical has picked up and allow accurate weighing. 3c. Dry for a minimum of 1 hour before proceeding to step 4. 3d. NOTE: Oven door should not be opened during drying period.	
	4. Transfer the container of potassium dihydrogen phosphate to a desiccator.	4a. Use tongs. 4b. NOTE: Potassium dihydrogen phosphate may be safely desiccated with ammonium persulfate. 4c. To cool to room temperature. 4d. About 30-40 minutes should be sufficient.	V.B.12.4 (p. 41)
	5. Transfer the container of potassium dihydrogen phosphate to a spot convenient to the analytical balance.	5a. Use tongs.	
	6. Weigh out exactly 0.2197 grams of potassium dihydrogen phosphate.	6a. In a weighing boat. 6b. On the analytical balance. 6c. NOTE: This step should be accomplished as quickly as is consistent with best weighing technique to avoid the pickup of atmospheric moisture by the chemical during weighing.	
	7. Completely transfer the 0.2197 grams of potassium dihydrogen phosphate to a one liter volumetric flask.	7a. Funnel the chemical into the flask, using a distilled water squirt bottle to wash all traces of the chemical from the weighing boat and funnel into the flask.	
	8. Fill the volumetric flask about one-half full.	8a. Use distilled water.	
	9. Gently swirl the flask.	9a. To completely dissolve the potassium dihydrogen phosphate.	
	10. Dilute the contents of the flask to one liter.	10a. Use distilled water.	

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of Orthophosphate (as P), Single Reagent Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	11. Stopper or cap the flask.		
	12. Gently invert the flask.	12a. Do this half-a-dozen times to ensure complete mixing.	
	13. Transfer the solution to a storage bottle.	13a. Bottle must be 1000 ml capacity or greater. 13b. Bottle can be glass or plastic.	
	14. Label the bottle "Stock Phosphorus Solution."	14a. 1.0 ml equals 0.05 mg P (50 microgram P). 14b. Solution is stable for a maximum of six months if stored at 4°C when not in use. 14c. NOTE: Solution must be warmed to room temperature before use.	
C. Preparation of Standard Phosphorus Solution	1. Pipet exactly 20 ml of stock phosphorus solution into a one liter volumetric flask.	1a. Use a 20 ml volumetric pipet and a rubber bulb. 1b. NOTE: This volume only applies for the Bausch and Lomb Spectronic 20 (or equivalent) equipped with the standard 1/2 inch tubes. For other than 1/2 inch tubes this volume must be adjusted. See Training Guide.	VI.C.1b (p. 42)
	2. Dilute the stock phosphorus solution in the flask to one liter.	2a. Use distilled water.	
	3. Stopper or cap the flask.		
	4. Gently invert the flask.	4a. Do this half-a-dozen times to ensure complete mixing.	
	5. Label the flask "Standard Phosphorus Solution."	5a. 1.0 ml equals 1.0 µg P. 5b. This dilute solution is unstable and must be prepared daily.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Preparation of Phosphorus Calibration Standards	1. If you are preparing a calibration curve, measure the amounts of standard phosphorus solution shown in Table 1 into nine 50 ml volumetric flasks. If a calibration curve has already been established, omit this step and steps 2 through 6 and proceed to step 7.	1a. Use volumetric pipets and a rubber bulb. 1b. Label each flask with its appropriate mg/l phosphorus concentration as given in Table 1. NOTE: If you will be using spectrophotometer tubes with a width greater than one half inch, the concentration of these standards will be different. See Training Guide. 1c. NOTE: The 40 ml volume of standard phosphorus solution may require a combination of volumetric pipets.	VI.D.1b (p. 42)

TABLE 1

Flask No.	ml of Standard Phosphorus Solution per 50.0 ml	Concentration of Phosphorus, mg per liter
1	0	0.00
2	1.0	0.02
3	3.0	0.06
4	5.0	0.10
5	10.0	0.20
6	20.0	0.40
7	30.0	0.60
8	40.0	0.80
9	50.0	1.00

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Preparation of Phosphorus Calibration Standards (continued)	2. Dilute the various amounts of standard phosphorus solution in the nine flasks to the 50.0 ml mark.	2a. Use distilled water. 2b. NOTE: The 50 ml flask requiring 0 ml of standard phosphorus solution is a "reagent blank" and will merely be filled to the 50 ml mark with distilled water. However, this flask must be carried through the rest of the steps, being treated exactly as any sample or calibration standard.	
	3. Stopper or cap each flask.		
	4. Gently invert each flask.	4a. Do this half-a-dozen times to ensure complete mixing.	
	5. Pour each of the nine prepared calibration standards from their 50 ml volumetric flasks into a 125 ml Erlenmeyer flask.	5a. Label each 125 ml Erlenmeyer flask with the mg/l P concentration corresponding to the particular 50 ml volumetric flask emptied into it.	
	6. If you are preparing a calibration curve, omit steps 7 through 15 and proceed to E, "Preparation of Samples."		
	7. Pipet 5 ml of standard phosphorus solution into a 50 ml volumetric flask.	7a. Use a 5 ml volumetric pipet and a rubber bulb.	
	8. Label the flask "0.10 mg/l P."	8a. This is a "low" calibration standard. It must be used to check the accuracy of the calibration curve.	
		8b. If you will be using spectrophotometer tubes with a width greater than one half inch, the concentration of this standard will be different. See Training Guide.	VI.D.8b (p. 43)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Preparation of Phosphorus Calibration Standards (continued)	9. Pipet 40 ml of standard phosphorus solution into a 50 ml volumetric flask.	9a. You may have to use a 20 ml volumetric pipet, filling it twice and using a rubber bulb.	
	10. Label the flask "0.80 mg/l P."	10a. This is a "high" calibration standard. It will be used to check the accuracy of the calibration curve.	
		10b. If you will be using spectrophotometer tubes with a width greater than one half inch, the concentration of this standard will be different. See Training Guide.	VI.D.10b (p. 43)
	11. Dilute the standard phosphorus solution in the two flasks to the 50.0 ml mark.	11a. Use distilled water.	
	12. Stopper or cap each flask.		
	13. Gently invert the flask.	13a. Do this half-a-dozen times to thoroughly mix the contents.	
	14. Empty these flasks into each of two 125 ml Erlenmeyer flasks.	14a. Label the 125 ml Erlenmeyer flasks with the corresponding mg/l P concentrations.	
	15. Pipet 50 ml of distilled water into a clean 125 ml Erlenmeyer flask.	15a. Use a 50 ml volumetric pipet and a rubber bulb.	
		15b. Label this flask "0.00 mg/l P."	
		15c. This is the "reagent blank." It is carried through all the steps, being tested exactly as any sample or calibration standard.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Preparation of Samples	1. Record the sample identification information.	1a. Sample should be at hand before continuing with this test.	VII.E.1a (p. 44)
		1b. Use a laboratory notebook.	IX.E.1b (p. 48)
		1c. Record "location," "identification," "type", "date and time collected," name of "sample collector," and "date and time analysis began" on the data sheet provided.	IX.E.1c (p. 49)
	2. Shake the sample.		
	3. Immediately pipet 50 ml of sample into a 125 ml Erlenmeyer flask.	3a. Use a 50 ml volumetric pipet and a rubber bulb unless the sample contains large particulate matter. Then use a 50 ml graduated cylinder.	
		3b. Measure rapidly since solids may settle in the sample container while you are filling the pipet or cylinder.	
		3c. NOTE: Wastewater samples may contain more than 1.00 mg/liter phosphorus and require dilution. With a wastewater sample of unknown mg/liter P concentration, it is desirable to set up additional flasks containing sample aliquots diluted to 50.0 ml.	VII.E.3c (p. 45)
		3d. NOTE: If orthophosphate is to be run, any sample containing appreciable quantities of turbidity or suspended solids must be filtered through a 0.45 micron phosphorus-free filter. Before attempting to run orthophosphate on such a sample, refer to the Training Guide for an explanation of the required procedure modification. Sample aliquots on which total phosphorus is to be determined must not be filtered at this time.	VII.E.3d (p. 46)
	4. Label this 125 ml Erlenmeyer flask "Sample."	4a. If the sample dilutions are being used, include the amount of dilution on the label.	II.E.4a (p. 40)
		4b. Also record the amount of sample dilution on the data sheet provided.	IX.E.4b (p. 49)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Preparation of Samples (continued)	5. If only orthophosphate is to be determined, adjust the pH of the sample and calibration standards to 7.0 ± 0.2, then skip Procedures F and G and start at Procedure H, "Preparation of Spectrophotometer." If total phosphorus is to be determined, continue with Procedure F, "Digestion Procedure for Total Phosphorus Determination."	5a. Use an electronic pH meter. 5b. Use the 10 N and 0.1 N sodium hydroxide and the strong acid solution (11 N sulfuric acid) and the 1.1 N sulfuric acid to adjust the pH. On any pH adjustment, begin with the strong acid (11 N) or base (10 N), and use the weaker (1.1 N sulfuric acid and 0.1 N sodium hydroxide) solutions only for the final precise adjustments. 5c. If no sample dilution is being used (i.e., you use 50.0 ml of sample) any acid or base used for pH adjustment will cause a volume error (final volume will be greater than 50.0 ml), and thus cause low results. Significant pH adjustment volume errors on strongly acid or basic samples may be minimized by very roughly adjusting the pH of the 50.0 ml aliquot using concentrated (36 N) sulfuric acid or very strong (10 N) sodium hydroxide dropwise, followed by precise adjustment using the more dilute solutions as given in 5b above. Small volume errors will still be unavoidable. 5d. If a sample dilution is being used, a volume error from pH adjustment may be avoided by pipetting the filtered sample aliquot into an Erlenmeyer flask or beaker, adding distilled water to bring the volume to approximately 40 ml, performing the pH adjustment, and then pouring the pH adjusted sample dilution into a 50.0 ml volumetric flask and adding distilled water as needed to bring the volume to the 50.0 ml mark. 5e. NOTE: If you are preparing a calibration curve, there will be nine calibration standards to pH adjust (prepared in D, steps 1 through 5). If a calibration curve has already been established, there will be three calibration standards to pH adjust (prepared in D, steps 7 through 15).	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Digestion Procedure for Total Phosphorus Determination (Calibration Standards, Reagent Blank, Samples)	1. Turn on a hot plate, or plates.	1a. Let them heat. 1b. The surface area of the hot plate(s) must be large enough to accommodate a minimum of 10-125 ml Erlenmeyer flasks. 1c. If an autoclave is to be used, omit this step.	
	2. Add 1 ml of strong acid solution (11 N sulfuric acid) to each 125 ml Erlenmeyer flask.	2a. Use a 10 ml graduated (Mohr) pipet and a rubber bulb. 2b. NOTE: All standards including the reagent blank are digested along with the sample.	
	3. Remove the ammonium persulfate from the desiccator.		
	4. Weigh out a 0.4 gram portion of ammonium persulfate for each solution in a flask.	4a. In weighing boats. 4b. Using a triple-beam balance. 4c. NOTE: If you are using a 0.4 gram Hach measuring spoon (or equivalent), this step may be omitted as the portions may be scooped as needed.	
	5. Add 0.4 gram ammonium persulfate to each of the 125 ml Erlenmeyer flasks.		
	6. Add 3 or 4 glass boiling beads to each flask.	6a. This will control bumping (uneven boiling).	
	7. Place the flasks on the preheated hot plate(s).	7a. Alternately, the flasks may be autoclaved for 30 minutes at 121°C (15-20 psi).	
	8. Gently boil the flasks.	8a. For 30-40 minutes or until a volume of approximately 10 ml is reached. 8b. CAUTION: Do not allow any of the flasks to go to dryness. This will ruin the determination.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. pH Adjustment of Digested Calibration Standards, Reagent Blank, Samples	1. Set up as many 0.45 micron filter assemblies as you have standards, blanks, and samples.	1a. See the Training Guide Note referenced to this step for information concerning this type of assembly and the required phosphorus-free filters. 1b. If you do not have this many filtration assemblies, you can rinse out and reuse the equipment. This requires more time.	VII.G.1a (p. 47)
	2. Cool the digestion flasks.	2a. Hold them under running tap water or use a very shallow cold-water bath.	
	3. Filter each standard, blank, and sample.	3a. Use a phosphorus-free 0.45 micron pore size filter and assembly.	
	4. Rinse each Erlenmeyer flask and filter the rinse water.	4a. Use distilled water. 4b. Use no more than 2,5 ml portions for each flask, adding each portion, swirling, and then pouring each portion through the appropriate filter.	
	5. Pour each filtrate back into its corresponding 125 ml Erlenmeyer flask.	5a. A powder funnel may be useful. 5b. For laboratories having only double-electrode pH meters, labeled 100 ml beakers may be substituted for the 125 ml Erlenmeyers at this point.	
	6. Rinse each filter flask.	6a. Use one 10 ml portion of distilled water, adding the rinse water to the 125 ml Erlenmeyer flasks. 6b. Volume in each flask must not exceed 35 ml.	
	7. Adjust the pH of each standard, blank, and sample.	7a. Adjust to pH 7 ± 0.2. 7b. Use an electric pH meter. 7c. NOTE: When adjusting the pH, add 10 N sodium hydroxide rapidly using a graduated (Mohr) pipet or eyedropper until the pH is raised to about 3 (this will require approximately 1 ml). Thereafter, add base slowly and dropwise to pH 6, watching the pH meter carefully. At this point continue the dropwise addition, but using 0.1 N sodium hydroxide until the pH is up to 7.0 ± 0.2. If pH is raised too high, use 1.1 N sulfuric acid dropwise to lower the pH.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. pH Adjustment of Digested Calibration Standards, Reagent Blank, Samples (continued)	8. Add 0.1 ml of 11 N sulfuric acid to each pH-adjusted standard, blank, and sample.	8a. Use a 1 ml pipet, graduated in 0.1 ml, and a rubber bulb. 8b. NOTE: This will prevent possible adsorption of phosphorus on iron, aluminum, manganese or other metal precipitates.	
	9. Pour each standard, blank, and sample into the 50 ml volumetric flask in which it was originally made up.	9a. If there is room in the 50 ml volumetric flask, add a small volume rinse of the flask or beaker used to adjust pH.	
	10. Dilute each flask to the mark.	10a. Use distilled water.	
	11. Stopper each flask.		
	12. Gently invert each flask.	12a. Do this half-a-dozen times to thoroughly mix the contents.	
	13. Pour each 50.0 ml solution back into its corresponding 125 ml Erlenmeyer flask.	13a. Each is now ready for the addition of colorimetry reagents.	
H. Preparation of Spectrophotometer	1. Turn the instrument on.	1a. Allow a warm-up period of approximately 20 minutes (10 minutes minimum). 1b. Use a B & L Spectronic 20 (or equivalent) equipped with accessory infrared phototube and filter for use at 880 or 650 nm wavelength. 1c. There is an EMP on "Use of a Spectrophotometer."	 V.H.1b (p. 41)

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of Orthophosphate (as P), Single Reagent Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Color Development	1. Add 8.0 ml of combined reagent to each 125 ml Erlenmeyer flask.	1a. Use a 10 ml graduated (Mohr) pipet and a rubber bulb.	
	2. Gently swirl the flasks.	2a. To ensure complete mixing.	
	3. Allow a 10 minute (minimum) to 30 minute (maximum) waiting period.	3a. For maximum color development.	
J. Spectrophotometric Measurements			
1. Adjusting the instrument	1. Consult the manufacturer's instructions for calibrating your particular instrument.	1a. Instrument must be warmed up at least 10 minutes. 1b. There is an EMP on "Use of the Spectrophotometer."	
	2. Adjust the wavelength to 880 nm.	2a. 880 nm is the preferred wavelength, but 650 nm may also be used.	V.J.1.2a (p. 41)
	3. Check to make sure that the instrument reads infinite absorbance with no sample tube in the instrument.	3a. If it does not, adjust the instrument so that it does read infinite absorbance. (See manufacturer's instructions).	
	4. Use the reagent blank (0.00 mg/liter P) to adjust the instrument to zero absorbance.	4a. Spectrophotometer tubes must be cleaned with 1:1 HCl, etc. See Procedure A, Glassware Preparation. 4b. Use manufacturer's instructions to make the adjustment.	
	5. Repeat step 3.		
2. Reading absorbance	1. Measure and record the absorbances for each of the calibration standards.	1a. If you are preparing a calibration curve, there are 8 calibration standards. 1b. If you are running check standards, there are 2.	

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Spectrophotometric Measurements (continued)		1c. In either case, proceed from the lowest to the highest concentration.	
		1d. Record the absorbance value next to the corresponding mg/liter P concentration of the calibration or check standards on the data sheet provided.	IX.J.2.1d (p. 49)
	2. Measure and record the absorbances for each of the samples.	2a. In the absorbance column provided for samples on the data sheet.	IX.J.2.2a (p. 49)
	3. Turn off the spectrophotometer.	3a. Unless it is to be used for other measurements.	
K. Making a Calibration Curve	1. If a calibration curve has been established, omit this Operating Procedure and proceed to Operating Procedure L, "Checking the Calibration Curve." If a calibration curve has not been previously established, proceed with step 2 below.	1a. Since the standards for a total phosphorus determination must be digested before colorimetry, and the standards for an orthophosphate determination are not digested, one calibration curve will be needed for the total phosphorus determination and a separate calibration curve for the orthophosphate determination. Unless they are exact duplicates, the two curves must not be used interchangeably.	
	2. Obtain an 8 1/2 x 11 inch piece of graph paper.	2a. The last page of this EMP is a model of graph paper labeled for this test.	IX (p. 50)
	3. Label the longer side as the concentration axis.		
	4. Label the shorter side as the absorbance axis.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
K. Making a Calibration Curve (continued)	5. Use the absorbance value and corresponding concentration for each of the standards to make a plot of absorbance versus concentration.	5a. This graph should be prepared with utmost care. 5b. The points plotted should form a straight line. 5c. This straight line plot is the calibration curve.	
	6. Skip Operating Procedure L, "Checking the Calibration Curve," and proceed to Procedure M, "Reading Results from the Calibration Curve."		
L. Checking the Calibration Curve	1. Locate the absorbance value just recorded for the 0.10 mg/liter P calibration standard.	1a. On the calibration curve for the determination you are doing--total phosphorus or orthophosphate. 1b. If you adjusted the concentration of your standards for other than half-inch width spectrophotometer tubes, the concentration of this standard is different. See Training Guide.	VI.L.1b (p. 42)
	2. Read its observed concentration.		
	3. Record this curve mg/liter P concentration.	3a. In the column next to the absorbance column for check standards on the data sheet provided.	IX.L.3a (p. 49)
	4. Compare this observed mg/liter P concentration to its true value of 0.10 mg/liter P.	4a. The observed mg/liter P concentration of the calibration standard, as read from the calibration curve must be within ± 2% of its true value of 0.10 mg/liter P. - 2% of 0.10 is 0.002 - Thus the acceptable range is 0.098 to 0.102 mg/liter P.	

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
L. Checking the Calibration Curve		4b. See Training Guide if you adjusted the concentration of this standard.	VI.L.4b (p. 43)
	5. If the observed concentration is within the acceptable range of the true value, proceed to step 6. If the observed concentration is not within the acceptable range of the true value, discard the calibration curve and prepare a new one by starting at Procedure D, following all directions for "If you are preparing a calibration curve."	5a. Failure of the observed and true concentrations to agree within ± 2% of the true value means that the calibration curve is no longer sufficiently accurate to report mg/liter P data obtained from it.	VII.L.5a (p. 47)
	6. Locate the absorbance value recorded for the 0.80 mg/liter P calibration standard.	6a. Again, on the calibration curve for your specific phosphorus determination. 6b. If you adjusted the concentration of the standards for other than half-inch width cells, use the adjusted concentration.	
	7. Read its observed concentration.		
	8. Record this curve mg/liter P concentration.	8a. In the column next to the absorbance column for check standards on the data sheet provided.	IX.L.8a (p. 49)
	9. Compare this observed mg/liter P concentration to its true value of 0.80 mg/liter P.	9a. The observed mg/liter P concentration of the calibration standard, as read from the calibration curve, must be within ± 2% of its true value of 0.80 mg/liter P. - 2% of 0.80 is 0.016 - The acceptable range is therefore 0.784 to 0.816 mg/liter P.	

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
L. Checking the Calibration Curve (continued)		9b. See Training Guide if you adjusted the concentration of this standard.	VI.L.9b (p. 43)
	10. If the observed concentration is within the acceptable range of the true value, proceed to Procedure M, "Reading Results from the Calibration Curve." If the observed concentration is not within the acceptable range of the true value, discard the calibration curve and prepare a new one by starting at Procedure D, following all directions for "If you are preparing a calibration curve."		
M. Reading Results from the Calibration Curve	1. Use the absorbance value recorded for each sample and the standard curve for your specific phosphorous determination to obtain the mg/liter P concentration.		
	2. Record this curve mg/liter P concentration.	2a. In the column next to absorbance column for samples on the data sheet provided.	IX.M.2a (p. 49)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
N. Calculations	1. Determine the dilution factor.	1a. The dilution factor for a straight (undiluted) sample is 1.	
		1b. The total sample volume is always 50 ml, so 25 ml of sample diluted to 50 ml in the volumetric flask would be 25/50 or 1/2 dilution, and the dilution factor would be 2. For other dilutions, see the Training Guide.	II.N.1b (p. 40)
		1c. The data sheet has a section with "Example Calculations."	IX.N.1c (p. 49)
	2. Record the dilution factor.	2a. In the column provided on the data sheet next to the curve mg/liter P column.	IX.N.2a (p. 49)
	3. Multiply the curve mg/liter P by the dilution factor.		
	4. Record this final mg/liter P.	4a. In the column provided on the data sheet.	IX.N.4a (p. 49)
	5. Sign the data sheet.	5a. On the line provided on the data sheet, "Analyst."	IX.N.5a (p. 49)
O. Reporting Data	1. Report total phosphorus, mg/liter P, or orthophosphate, mg/liter P.	1a. On any required record or report sheets.	IX.O.1a (p. 48)
P. Clean-Up	1. Discard unused combined reagent and standard phosphorus solution.	1a. Combined reagent must be made fresh before each run. 1b. Standard phosphorus solution may be retained for other analyses to be performed that same day.	
	2. Store the other reagents.	2a. Observe special storage requirements of some reagents as stated in B, "Reagent Preparation."	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
P. Clean-Up (continued)	3. Transfer all glassware to wash area.		
	4. Clean all glassware.	4a. In readiness for next determination. 4b. According to the steps in Operating Procedure A, "Glassware Preparation." 4c. This step may be performed when time permits. 4d. It is desirable, but not mandatory, that all glassware used in this procedure be maintained as a separate stock, used only for the phosphorus determination. 4e. NOTE: Never clean glassware to be used in phosphorus determinations in commercial detergent, as the active ingredient is usually a phosphate compound.	

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of Orthophosphate (as P), Single Reagent Method

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI*	Field & Laboratory Reagents
VII*	Field & Laboratory Analysis
VIII	Safety
IX*	Records & Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

INTRODUCTION

Section I

TRAINING GUIDE NOTE	REFERENCES/RESOURCES
Sources of phosphates in water besides the geological include agricultural fertilizers, sewage (human wastes, synthetic detergents, biological protoplasm) and various industrial wastes.	Standard Methods for the Examination of Water and Wastewater. 14th ed., 1976 APHA, New York, N.Y. p. 466
Phosphates are a necessary and sometimes growth-limiting nutrient for microorganisms. In high concentrations, phosphates can produce nuisance levels of algae and other photosynthetic aquatic organisms.	Griffith, et. al., editors. Environmental Phosphorus Handbook. 1973. John Wiley and Sons, New York, N.Y. p. 443ff.
Since the natural phosphorus content of most waters is quite low, the presence of high phosphate concentrations can be an excellent indicator of the level of pollution. Hence, the phosphate test will be a common tool of technicians monitoring water quality for the NPDES system.	
The orthophosphate $(PO_4)^{\equiv}$ ion is the smallest and simplest of the phosphorus-oxygen radicals. The orthophosphate determination as given here is limited to the inorganic phosphorus $(PO_4)^{\equiv}$ in the sample as measured by the direct colorimetric analysis procedure.	Methods for Chemical Analysis of Water and Wastes. 1974. EPA, MDQARL, Cincinnati, OH 45268. p. 251.
More complex phosphorus compounds are usually composed of linked orthophosphates or of phosphorus linked to carbon compounds (organic phosphorus). The total phosphorus determination as given here refers to all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure.	
The test described in this instruction can be found in the 1974 EPA Methods Manual on page 249, titled Phosphorus, All Forms (Single Reagent Method). Other references which have acceptable procedures for NPDES purposes are: 14th ed. Standard Methods, on pages 476 and 481, and 1975 ASTM Part 31 on page 384.	Ibid, p. 249. Op. cit. pp 476 and 481. Annual Book of Standards, Part 31, Water, 1975, ASTM Philadelphia, PA, p. 384.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.4a N.1b	Since the dilution is only part sample, when the absorbance reading obtained for it is converted to a mg/liter P concentration using the calibration curve, the concentration obtained is only that of the dilution. To obtain the mg/liter P concentration of the sample, the mg/liter P concentration of the dilution must be multiplied times the amount of dilution factor. For a 1/2 dilution (25 ml sample/50 ml total volume) the dilution factor would be 2 (the dilution is only half sample). For a 1/5 dilution (10 ml of sample/50 ml total volume) the dilution factor would be 5. Use of dilution factors is illustrated for a total phosphorus determination in the typical data sheet in Section IX at the back of this Training Guide. Below is a table of common dilution factors for a 50 ml sample.	

ml of Sample per 50 ml Total Volume	Amount of Dilution	Dilution Factor
25	1/2	2
10	1/5	5
5	1/10	10
1	1/50	50
0.5	1/100	100
0.05	1/1000	1000

The dilution factor for any dilution may be calculated by dividing the ml of sample used in the dilution into 50:

$$\text{Dilution Factor} = \frac{50\text{ ml}}{\text{ml sample used in dilution}}$$

Example: 2 ml of sample diluted to 50 ml

$$\text{Dilution Factor} = \frac{50\text{ ml}}{2\text{ ml}} = 25$$

The dilution factor would be 25.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.11.2a B.12.4	Desiccants are hygroscopic materials capable of absorbing moisture from air. Silica gel (SiO_2) and calcium sulfate ($CaSO_4$) are two commonly used desiccants available from laboratory supply companies. Desiccants must always be dry before use. The moisture can be removed from them by heating in an oven (103-105°C).	
H.1b J.1.2a	Ordinarily a wavelength of 880 nm is used for phosphorus determinations. The second wavelength (650 nm) may be desirable because of your particular instrument capabilities or because of unusual interferences in the sample. If you have such a situation, test your standards at the 650 nm wavelength to see if you get a range of responses significant enough to construct a calibration curve. If you do, you can use the 650 nm wavelength setting.	

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

C.1b
D.1b
L.1b

The mg/liter P concentration range that a Spectronic 20 with 1/2 inch colorimeter tubes can detect is from 0.02 mg/liter P to approximately 1.00 mg/liter P. This covers the useful working range of absorbance readings from 0.005 to approximately 0.7 (Readings above about 3/4 of full scale deflection, which is approximately 0.7 absorbance, are inaccurate and should be discarded). Using 20 ml of stock to prepare 1 liter of standard phosphorus solution allows the preparation of 8 calibration standards whose mg/liter P concentration covers the range of 0.02 to 1.00 mg/liter P. If the absorbance of these same solutions were to be measured in 1 inch colorimeter tubes, they would give absorbances ranging from 0.02 to approximately 1.4. Since the useful working range is from 0.005 to about 0.7, about half of the standards would be useless, as they would read off the scale. This is because with a 1 inch colorimeter tube, you are measuring the absorbance of twice the thickness of colored solution, and twice the thickness of a given colored solution will absorb twice as much light and give twice the absorbance reading (Beer's Law).

If you are using 1 inch colorimeter tubes, you will need to use 10 ml of stock phosphorus solution, rather than 20 ml, to prepare the standard phosphorus solution. The various ml of standard phosphorus solution used in Table 1 (Operating Procedure D.1c) will then give calibration standards of the correct concentrations for use with 1 inch colorimeter tubes. The concentrations as given in Table 1 will now be inaccurate, however. If you use 1 inch spectrophotometer tubes, and hence only use 10 ml of stock phosphorus solution to make up the standard phosphorus solution, Table 1 will read as follows:

ml of Standard Phosphorus Solution per 50.0 ml	Concentration of Phosphorus, mg/liter
0	0.00
1.0	0.01
3.0	0.03
5.0	0.05
10.0	0.10
20.0	0.20
30.0	0.30
40.0	0.40
50.0	0.50

(continued)

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.1b D.1b L.1b (continued)	Notice that since you are using 1 inch colorimeter tubes that have twice the thickness of 1/2 inch tubes, the mg/liter P concentrations of the standards have been halved. Calibration curves and data sheets made up using these 1/2 strength standards will need to have these new concentrations substituted for those given in the typical calibration curve and data sheet at the back of this Training Guide, as they are examples of data obtained using 1/2 inch colorimeter tube calibration standards.	
D.8b D.10b L.4b L.9b	If you are using 1 inch colorimeter tubes, the strength of the calibration curve check standards will also be different, and hence the acceptable range of observed concentrations they can have will be different. For 1 inch tubes the concentration of the calibration curve check standard using 5 ml of that standard phosphorus solution will be 0.05 mg/liter P. Two percent of 0.05 is 0.001, so the acceptable ± 2% range will be from 0.049 to 0.051 mg/liter P. The concentration of the calibration curve check standard using 40 ml of standard phosphorus solution will be 0.40 mg/liter P. Two percent of 0.40 is 0.008, so the acceptable observed concentration range will be 0.392 to 0.408 mg/liter P.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.1a	COLLECTION OF SAMPLES FOR THIS TEST:	
	Samples should be collected from a preagreed site by a preagreed technique known to all parties concerned. You should be familiar with the following information since you record most of it on your laboratory data sheet. You may be responsible for actually collecting the sample; consult your supervisor.	
	LOCATION - Plant control and self-monitoring requirements will be the basis for selecting places to collect samples. Final collection points should be such that samples drawn there are as representative of the entire sample source as possible. Consult your supervisor.	Standard Methods for the Examination of Water and Wastewater. 14th ed., 1976, APHA, New York, NY, p. 38.
	IDENTIFICATION - Each collection location should be assigned a number or simple identification code. Use this to label samples from that location and to record on the lab data sheet.	
	TYPE - Permit requirements determine whether a grab or a composite sample will be collected; consult your supervisor. Mark type on sample container and on laboratory data sheet.	Ibid.
	TIME OF COLLECTION - Mark time and date on sample container and on lab data sheet.	
	CONTAINER - The analyst should know what volume container is required for each sample source. Containers should be capped, and may be of plastic material (such as cubitainers) or of Pyrex glass. Used containers should be rinsed with hot 1:1 HCl, with tap water (2 times), with distilled water; checked for phosphate traces with combined reagent, then rinsed again with tap and distilled water (see Operating Procedure A, "Glassware Preparation," in the EMP for specific details).	Methods for Chemical Analysis of Water and Wastes. 1974. EPA-NERC-MDQARL, Cincinnati, Ohio 45268. p. 249.
	COLLECTION - Rinse container two or three times with sample, then collect the sample. If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.	Ibid.
	SIGNATURE - Sample collector should sign his name on the container or label so this information can be recorded on the lab data sheet.	
	(continued)	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.1a (continued)	PRESERVATION - If the analysis cannot be performed the same day as collection, the sample should be preserved by the addition of 2 ml concentrated sulfuric acid (H_2SO_4) or 40 mg mercuric chloride ($HgCl_2$) per liter and refrigeration at 4°C. If $HgCl_2$ is used as a preservative, samples should be spiked with a minimum of 50 mg/liter of sodium chloride (NaCl) to prevent interference of the $HgCl_2$ with samples containing low (less than 50 mg Cl/l) chloride levels.	Ibid, p. 249-50, 252.
	HOLDING TIME - Maximum holding time for preserved samples is seven days. Samples for the orthophosphate determination that must be filtered, should be filtered as soon as practical after collection.	Ibid, p. x and xi
E.3c	A phosphorus determination on a 50 ml aliquot of any sample containing over 1.00 mg/liter P will result in an absorbance outside the range of the calibration curve. The blue color produced by addition of the combined reagent will be so strong that the spectrophotometer will be unable to measure it. Samples containing over 1.00 mg/liter P concentrations must be diluted. Since the mandatory sample size is 50 ml, all dilutions will be based on a lesser amount of sample diluted to 50 ml. The correct procedure is to use a volumetric pipet to transfer a volume of sample to a 50 ml volumetric flask, then to dilute that volume of sample to 50 ml with distilled water and mix thoroughly. This dilution may then be used in the procedure. A natural question arising is, "what amount of dilution should I use?" The best answer is that only trial and error experience will show you the best dilution to use with a given sample. A rule of thumb is that potable water samples will usually require little or no dilution. A typical series to run on a potable water sample of unknown mg/liter P concentration might be to prepare one flask containing 50 ml of undiluted sample, one flask containing 25 ml of sample diluted to 50 ml (this would be a 1/2 dilution, 25 ml sample/50 ml total volume) and a third flask containing 10 ml of sample diluted to 50 ml (this would be a 1/5 dilution, 10 ml sample/50 ml total volume).	
	(continued)	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.3c (continued)	Sewage samples may contain 10 mg/liter P concentrations or more, and consequently require dilutions as high as 1/1000 (.05 ml of sample diluted to 50 ml). The problem encountered here is that volumes of less than 1 ml are hard to measure directly with any accuracy, and obtaining a representative sewage sample using such a small volume is unlikely. In making dilutions requiring less than 1 ml of sample, a good procedure is to use dual dilutions. A dual dilution means taking a volume of sample, diluting it, taking a volume of the first dilution, and diluting it again. To illustrate the use of dual dilutions, consider a sample requiring a 1/100 dilution to get a mg/liter P concentration inside the required range of 0.02 mg/liter to 1.00 mg/liter. First, take a 50 ml volumetric flask and pipet into it 5 ml of sample. Dilute this flask to the mark, and you have a 1/10 dilution. Each 1 ml of the contents of this flask contains 0.1 ml of the original sample. 0.5 ml of the sample is needed to dilute to 50 ml to achieve a 1/100 dilution, so if you pipet 5 ml from the first dilution flask into a second 50 ml volumetric flask, you will have 0.5 ml of sample in a 50 ml flask. When diluted to the 50 ml mark, this second flask will be a 1/100 sample dilution, ready for determination.	
E.3d	INTERFERENCES - Turbidity or suspended solids interfere with the orthophosphate determination. This is because a spectrophotometer works by measuring the amount of light absorbed by color produced in a sample by the addition of specific reagents. Turbidity or suspended solids in a sample will falsely increase the absorbance reading because a spectrophotometer cannot differentiate between light absorbed by the color in a sample and that scattered or blocked by solids.	
	The interference of turbidity or suspended solids in the orthophosphate determination may be eliminated by filtering the sample through a .45 micron membrane filter before the determination is begun. The filtration should be done as soon as possible after sample collection. A change in the name of the phosphorus fraction reported must be made to signify that the sample was filtered. An orthophosphate determination made on a filtered sample must be reported as Dissolved Orthophosphate, mg P/liter.	Ibid., p. 251.
	(continued)	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.3d (continued) G.1a	A membrane filter assembly typically consists of a funnel clamped to a fritted (porous) base, holding between them a .45 micron pore size cellulose membrane filter. A stopper on the fritted base is used to hold this assembly upright in the neck of a 500 ml side-arm flask connected to a vacuum source. A quantity of well-mixed sample is measured into the funnel. The vacuum is applied, and the sample is drawn through the filter into the side-arm flask. This filtered sample will now be free from all turbidity or suspended solids. It may be decanted from the side-arm flask and used in subsequent steps of the determination.	
	Since the very fine porosity filters clog quickly, samples containing high levels of particulate matter may require that 2 or 3 filters be used in succession to obtain enough filtrate for the determination. In the case of a total phosphorus determination, the entire digested sample must be filtered and recovered.	
	Before use, the membrane filter assemblies must be cleaned in the same manner as all other glassware used in the procedure.	
	The membrane filters must also be phosphorus-free. This can be accomplished by soaking ordinary .45 micron membrane filters in distilled water: 50 filters per 2 liters distilled water for 1 hour, changing the water, and soaking an additional 3 hours. Alternately, phosphorus-free filters may be purchased (Gelman GA6 or equivalent).	Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, NY. p. 472.
	In the determination of total phosphorus, low values have been reported because of possible adsorption of phosphorus on iron, aluminum, manganese or other metal precipitates. This can be avoided by filtration before neutralization and re-dissolving the metal hydroxides that form with 2-3 drops of acid before color development.	"Changes and Errata in Methods for Chemical Analysis of Water and Wastes," 1974, EPA-NERC-MDQARL, Cincinnati, Ohio 45268
L.5a	If you find that you must frequently discard your calibration curve because one or both of the 0.10 and 0.80 mg/liter P calibration curve check standards fall outside the ± 2% acceptable range of their true value, you may find it advisable to run the full set of calibration curve standards and prepare a new calibration curve for each batch of samples determined.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.1b	All laboratory records must be kept for three years, preferably in a permanently bound notebook. The time period is required by regulatory agencies. Attached as the next two pages are an example data sheet and a graph which can be used to construct a calibration curve. These can be used for either a Total Phosphorus (as P) or an Orthophosphate (as P) determination.	
O.1a	Depending on your organizational set-up, it may be your job responsibility to enter this data on the plant operation record, state report form, etc. Check with your supervisor.	

EXAMPLE DATA SHEET FOR TOTAL PHOSPHORUS OR FOR ORTHOPHOSPHATE, mg/liter P

E.1c	Sampling Location	Final Effluent	
E.1c	Sample Identification	E.S. 1s.	
E.1c	Type of Sample	Grab or Composite	
E.1c	Date and Time Collected	1/17/75 9:00 a.m.	
E.1c	Sample Collector	Tom Sampler	
E.1c	Date and Time Analysis Began	1/17/75 9:30 a.m.	
N.5a	Analyst	Dick Analyst	

J.2.1d L.3a L.8a	Calibration Standards mg/liter P	Absorbance	Check Standards mg/liter P	Absorbance	Curve mg/liter P
	0.02				
	0.06				
	0.10		0.10		
	0.20				
	0.40				
	0.60				
	0.80		0.80		
	1.00				

E.4b J.2.2a M.2a N.2a N.4a	Amount of Sample Dilution	Absorbance	Curve mg/liter P	Dilution Factor	Final mg/liter P

EXAMPLE CALCULATIONS

N.1c	Amount of Sample Dilution	Absorbance	Curve mg/liter P	Dilution Factor	Final mg/liter P
	Straight Sample	off scale	-	1	-
	$\frac{1}{2}$ (25 ml sample / 50 ml total)	off scale	-	2	-
	$\frac{1}{5}$ (10 ml sample / 50 ml total)	0.3525	0.520	5	2.60
	$\frac{1}{10}$ (5 ml sample / 50 ml total)	0.1775	0.260	10	2.60
	$\frac{1}{20}$ (2.5 ml sample / 50 ml total)	0.0875	0.130	20	2.60

EFFLUENT MONITORING PROCEDURE: Determination of Total Phosphorus (as P) or of Orthophosphate (as P), Single Reagent Method

RECORDS AND REPORTS

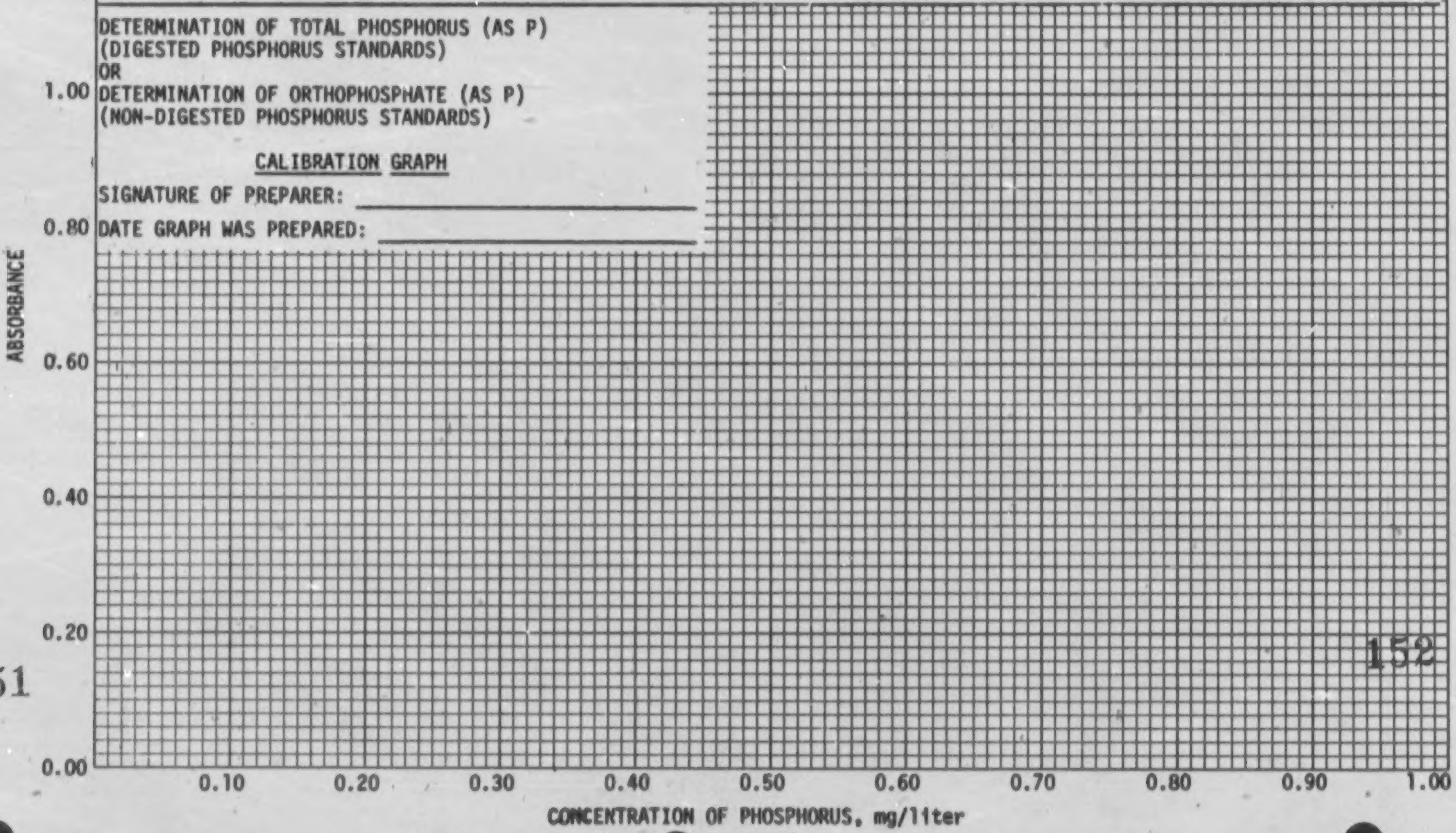
SECTION IX

DETERMINATION OF TOTAL PHOSPHORUS (AS P)
(DIGESTED PHOSPHORUS STANDARDS)
OR
DETERMINATION OF ORTHOPHOSPHATE (AS P)
(NON-DIGESTED PHOSPHORUS STANDARDS)

CALIBRATION GRAPH

SIGNATURE OF PREPARER: ______________________________

DATE GRAPH WAS PREPARED: ____________________________



A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF CHEMICAL OXYGEN DEMAND

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. ENVIRONMENTAL PROTECTION AGENCY

EFFLUENT MONITORING PROCEDURE: Determination of Chemical Oxygen Demand

This operational procedure was developed by:

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Education and Technical Background

B.A. Edgecliff College
1 year Industrial Research Chemist
8 years Secondary School Chemistry Instructor
4 years DHEW-DI Water Quality Program Chemist
6 years DI-EPA Chemist-Instructor

EFFLUENT MONITORING PROCEDURE: Determination of Chemical Oxygen Demand

1. Objective:

   To determine the mg/liter Chemical Oxygen Demand of organic and oxidizable inorganic substances in a wastewater sample.

2. Description of Analysis:

   A measured water sample is mixed with a measured volume of potassium dichromate solution which is a strong oxidizing agent. A volume of concentrated sulfuric acid equal to the combined volume of sample and oxidizing agent is added to provide a 50% by volume mixture which particularly promotes oxidation of organic and oxidizable inorganic substances in the sample.

   The mixture is in a flask which is then attached to a condenser over a source of heat. The heat is applied to maintain the mixture at a gentle boiling temperature of 145°C for a two hour period. The condenser cools and reliquifies materials that vaporize during this period.

   In order to determine the amount of sample that is oxidized under these conditions, the potassium dichromate solution must be added in excess. The measurement involves titrating any unused oxidizing solution after the oxidation period, and then calculating the Chemical Oxygen Demand from the amount of oxidizing solution that was used. A reducing agent, ferrous ammonium sulfate solution, is used to titrate the unused potassium dichromate solution in the test mixture. Ferroin is used as a color indicator in this titration.

   If there is no potassium dichromate left to titrate after the two hour oxidation period, the test must be done over using less sample. Water is added to make up for the missing volume of sample in order to maintain the 50% volume of concentrated sulfuric acid required in the test mixture.

   Organic substances are particularly susceptible to oxidation when placed in the conditions of this test. Even when the best laboratory technique is used, some organic contamination may be present and will affect test results. Consequently, a blank using distilled water instead of sample is run with each group of samples and is titrated with ferrous ammonium sulfate solution. The results are included in the calculation formula to correct the data for minor contamination. The titration results for the blank may be of a magnitude to prompt a check of reagents and/or distilled water as contributors of excessive organic contamination in the test.

3. Applicability of this Procedure:

   a. Range of Concentration:

      5 to 50 mg/liter COD
      Information is given so the same stepwise procedure can be used for COD greater than 50 mg/liter.

   b. Pretreatment of Samples:

      The Federal Register Guidelines do not specify any pretreatment.

c. Treatment of Interferences in Samples:

This procedure includes directions for conditioning glassware and information about checking distilled water to minimize organic contamination. To minimize loss of volatile materials during the addition of sulfuric acid, instructions include cooling the test flask in ice water. Addition of mercuric sulfate to complex routine levels of interfering chlorides is also part of the procedure. However, if the chloride concentration exceeds 2000 mg/liter, consult the Source of Procedure* for the required modification of mercuric sulfate addition and of the calculation formula.

No other interferences are noted in the Source of Procedure.*

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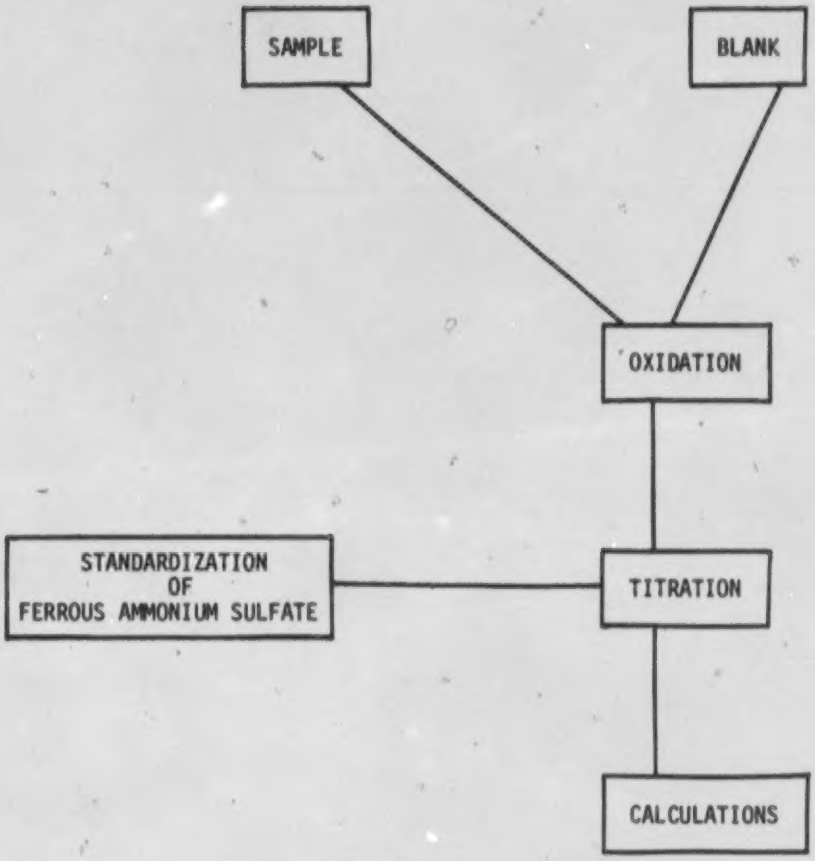
*Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection AGency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, OH, p. 21.

EFFLUENT MONITORING PROCEDURE: Determination of Chemical Oxygen Demand

FLOW SHEET:


SAMPLE
BLANK
OXIDATION
STANDARDIZATION OF FERROUS AMMONIUM SULFATE
TITRATION
CALCULATIONS

EFFLUENT MONITORING PROCEDURE: Determination of Chemical Oxygen Demand

Equipment and Supply Requirements

A. Capital Equipment:

1. Balance, with a 0.1 or 0.01 gram sensitivity

2. Balance, analytical with a 0.1 milligram sensitivity

3. Distillation Equipment - Use an all-glass distillation unit if possible. A metal still is acceptable if all the surfaces that contact the distillate are heavily coated with pure tin. The still should be located away from areas where volatile organic solvents are stored and/or used. DO NOT USE ion - exchange columns or membrane filters to prepare the water. These treatments can add organic contamination.

4. Magnetic Stirrer - Hot Plate and Magnetic retriever (pick-up rod). OPTIONAL

5. Oven, laboratory for drying chemicals at 103°C.

6. Specific Conductance Meter and related equipment to test inorganic quality of distilled water. OPTIONAL

7. Total Organic Carbon Analyzer and related equipment to test organic quality of distilled water. OPTIONAL

B. Reusable Supplies:

1. Reflux Apparatus: One flask-condenser-heating surface assembly is required for each sample or blank to be tested. These should be permanent assemblies in the laboratory, protected from contamination by glass wool plugs in the open end of the condensers and with the flasks connected to the condensers.

   Flasks, heat-resistant glass, 500 ml Erlenmeyer or 300 ml round bottom, with a ground glass neck to fit the condenser of choice. If the Erlenmeyer type flask is to be used, purchase those having graduations for approximate volumes contained in the flask.

   Condensers, 12 inch Allihn or equivalent with a ground glass joint to fit into the flask. (24/40 is a commonly used joint size.)

   Tubing Connections from cooling water source to condensers.

   Heating Surface, flat for Erlenmeyer flasks or heating mantles for round bottom flasks. Either should have sufficient power to produce at least 9 watts/square inch to supply the 145°C temperature required. The amount of heat supplied should be adjustable.

   NOTE: A 16 amp line is usually required for a series of 6 reflux set-ups.

2. 2 Automatic dispensers (pipets), glass with delivery settings up to 10 ml. OPTIONAL

3. Beads, glass about 2 mm diam. 5 for each flask - condenser assembly

B. Reusable Supplies: (Continued)

4. 1 Beaker, glass, 250 ml
5. 2 Bottles, brown glass, about 50 ml with dropper pipet in screw cap for ferroin. Alternatively, use a stoppered reagent bottle and a medicine dropper.
6. 3 Bottles, glass, screw cap, minimum capacity of 1 liter each to store reagents.
7. 1 Buret, 50 ml, 0.1 ml graduations, teflon stopcock plug preferred.
8. 1 Clamp, buret, for titration stand
9. Containers, storage, glass or heavy plastic with screw caps for COD waste test materials containing mercury complexes and significant amounts of sulfuric acid.
10. 1 Buchner funnel to catch glass beads when test wastes are transferred from flasks to storage containers.
11. 2 Cylinders, graduated, 25 ml.
12. 2 Cylinders, graduated, 100 ml.
13. 1 Cylinder, graduated, 500 ml.
14. 1 Desiccator to store cooling chemical for reagent preparation.
15. 1 Evaporating dish per sample to separate flask from heating surface. (Optional)
16. 2 Flasks, Erlenmeyer, wide mouth 500 ml.
17. 3 Flasks, volumetric, 1 liter.
18. 1 Funnel, short stem, diam. about 75 mm (to fill 50 ml buret).
19. 1 Pan for ice water to cool mixtures, about 4 inch depth and about 8 inch diameter is sufficient.
20. 1 Pipet bulb
21. 1 Pipet, graduated, 10 ml (Omit if an automatic dispenser is used for the concentrated sulfuric acid).
22. 1 Pipet, volumetric, 10 ml.
23. 1 Pipet, volumetric, 25 ml.
24. 2 Pipets, volumetric, 50 ml.
25. 2 Pipets, volumetric, 100 ml.
26. 1 Reagent bottle, glass with glass stopper. Only required if preparing less than 9 pounds of the sulfuric acid - silver sulfate solution.
27. 1 Reagent spoon to roughly measure 1 gram of mercuric sulfate.
28. Rings, cork as supports if round bottom flasks are used, 1 per flask.
29. 1 Stand, titration, support for buret.

B. Reusable Supplies: (Continued)

30. 1 Stirring rod, glass to use in 250 ml beaker. Omit if ferroin solution is purchased already prepared or if a magnetic stirrer is available.

31. Storage containers for distilled water, preferably glass. If only polyethylene bottles are available, be aware that organic plasticizers may be leached into water stored in such bottles over a period of time.

32. 1 Wash bottle, squeeze type 500 ml.

C. Consumable Supplies:

1. Glass wool, to make plugs for condensers, bottle of distilled water, etc.

2. Labels for reagent bottles, at least 7.

3. Laboratory notebook with spaces for information similar to the "Typical Laboratory Data Sheet" in this EMP.

4. Pencil, wax marking.

5. Towels, paper.
6. Weighing boats, at least 5.
7. Ice to cool flasks during test.
8. Reagents - Quantities for one sample plus one blank:

   2 grams mercuric sulfate ($HgSO_4$) reagent grade.

   1 1/3 - 9 pound bottles concentrated sulfuric acid (H_2SO_4) reagent grade.

   23.5 grams silver sulfate (Ag_2SO_4) reagent grade

   6.5 liters distilled water, high quality with very low chemical oxygen demand

   14 grams potassium dichromate ($K_2Cr_2O_7$) primary standard grade.

   *1.5 grams 1-10 (ortho) phenanthroline with one molecule of water of hydration (ferroin) ($o\text{-}C_{12}H_8N_2 \cdot H_2O$).

   *1 gram ferrous sulfate with seven molecules of water of hydration ($Fe\,SO_4 \cdot 7H_2O$)

   98 grams ferrous ammonium sulfate with six molecules of water of hydration [$Fe\,(NH_4)_2(SO_4)_2 \cdot 6H_2O$]

*If ferroin indicator solution is purchased, these reagents are not required.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Preparing to Test the Sample.	1. Assemble all equipment to be used.	1a. Equipment list is on pp.6, 7 and 8. 1b. Flasks, boiling beads and condensers should be with the chosen heat source in a permanent assembly.	I (p. 45)
	2, Prepare the reagents for the test.	2a. See Procedure B. Reagent Preparation	
	3. If necessary, condition any flasks, boiling beads or condensers to be used for the test.	3a. Conditioning is necessary if the equipment is new, if it has been used for COD mixtures that turned green during the boiling period, or if it was used for tests other than COD. 3b. See Procedure D, "Conditioning Flasks, Boiling Beads and Condensers"	
	4. If necessary, condition any other glassware to be used in the test.	4a. This glassware is included in the equipment list on pp. 6, 7 and 8. 4b. Conditioning is necessary if the glassware is new, if it has been used to measure COD samples, or if it has been used for tests other than COD. 4c. See Procedure E, "Conditioning Glassware Other Than Flasks, Boiling Beads or Condensers".	
	5. Record the sample identification information.	5a. The sample should be at hand before continuing with the test.	
		5b. Use a laboratory notebook with space for information similar to the "Typical Laboratory Data Sheet" in this EMP.	IX.Sheet I (p. 51)
		5c. Record "Identification", "Type"(grab or composite), "Date and Time Collected", and the name of the "Sample Collector" in one of the columns.	IX.A.5. (p. 51)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation			
1. Mercuric Sulfate	1. Use a 1 gram reagent spoon to measure the mercuric sulfate ($HgSO_4$) at the time of the test.	1a. Use reagent grade powdered mercuric sulfate. 1b. Use one gram for each sample and for the blank.	
2. Concentrated sulfuric acid	1. Use concentrated sulfuric acid (H_2SO_4) to prepare other reagents and also as a reagent in the test.	1a. You need reagent grade concentrated sulfuric acid. 1b. You need 2.5 liters for preparations. 1c. You need 5 ml for each sample and for the blank. 1d. Since sulfuric acid causes severe burns to the skin, you may want to put it in an automatic dispenser for use during the test. Label the dispenser.	V.B2.1d. (p. 46)
3. Sulfuric acid - silver sulfate solution	1. In a weighing boat, weigh 23.5 grams of silver sulfate (Ag_2SO_4).	1a. Use reagent grade silver sulfate. 1b. You need 70 ml of this solution for each sample and each blank. If you do this test routinely, it is easiest to prepare the amount of reagent as given in this procedure. To make smaller volumes of the reagent, multiply the ml of reagent desired by 0.0108 grams to find how many grams of silver sulfate are needed. 1c. Use a balance with 0.1 or 0.01 gram sensitivity.	
	2. Put the weighed chemical in a 9 pound bottle of reagent grade, concentrated sulfuric acid.	2a. To make smaller volumes, measure the acid with a graduate and carefully pour it into a glass reagent bottle. Add an amount of silver sulfate calculated as described above in 1b.	
	3. Screw the cap onto the bottle of acid.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
3. Sulfuric acid - silver sulfate solution (continued)	4. Swirl the mixture in the bottle every half hour or so until the silver sulfate dissolves.	4a. It will take several hours for the silver sulfate to dissolve. If you have a magnetic stirrer assembly, use it to speed up the dissolving. CAUTION: Sulfuric acid causes severe skin burns. Be careful not to splash it out of the bottle when you put the stirring bar in. Also, use a retriever to get the bar out and thoroughly rinse the acid off of the retriever and the stirring bar at once, with water.	
	5. Label the container.	5a. This is the sulfuric acid - silver sulfate solution to be used in the test. Also write the date and your name on the label.	
		5b. You may want to put some of this solution in an automatic dispenser for use during the test. Label the dispenser.	V.B3.5b. (p. 46)
4. Distilled water	1. Prepare 7 liters of high quality distilled water with very low chemical oxygen demand due to organic or inorganic contamination.	1a. Requirements for distillation equipment are described on p. 6.	
		1b. When distilling water, use clean glass wool packing around delivery tubes to prevent organic contamination of the distillate.	
		1c. Requirements for water storage containers are described on p. 8.	
		1d. Mark the date of distillation on the water container.	
		1e. Plug the container of distilled water with clean glass wool or cover it with a screw cap.	
		1f. Store the container of distilled water away from areas where organic solvents are stored and/or used.	
		1g. You can test the inorganic quality of water with specific conductance measurements, either in line or on the distillate. The specific conductance should be less than 2.0 micromhos at 25°C.	

(continued)

EFFLUENT MONITORING PROCEDURE: Determination of Chemical Oxygen Demand

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
4. Distilled water (continued)		1h. The organic quality of water is difficult to monitor in line. If test blanks indicate significant organic contamination (See Training Guide, BLANKS) you could arrange to have total organic carbon tests done on the distillate. At least check the still for cleanliness and check storage procedures.	VII.B4.1h. (p. 48)
5. 0.250 N potassium dichromate solution	1. Dry about 14 grams of potassium dichromate ($K_2Cr_2O_7$) in a laboratory oven for two hours at 103°C.	1a. Use primary standard grade potassium dichromate. 1b. A round weighing is sufficient for this step.	
	2. Remove the chemical from the oven to a desiccator to cool.	2a. Desiccant should be dry. 2b. Allow about 20 minutes for cooling.	
	3. In a weighing boat weigh out 12.259 grams of the dried potassium dichromate.	3a. Use an analytical balance.	
	4. Put the weighed chemical into a 1 liter volumetric flask.		
	5. Add about 500 ml distilled water to the flask.	5a. Use high quality distilled water with very low COD (See B.4).	
	6. Swirl to dissolve the potassium dichromate.	6a. Support the bottom of the flask with your hand while swirling.	
	7. Add distilled water up to the one liter mark on the flask.		
	8. Mix the solution by inverting the flask several times.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
5. 0.250 N potassium dichromate solution. (continued)	9. Pour the solution into a screw cap bottle.		
	10. Label the container.	10a. This is 0.250 N potassium dichromate solution. It is used for testing samples with COD greater than 50 mg/liter. 10b. It is very stable and can be stored at room temperature for several months. 10c. To use it for COD less than 50 mg/liter, you must dilute it to be 0.025 N.	
6. 0.025 N potassium dichromate solution.	1. Measure 100.0 ml of 0.250 N potassium dichromate ($K_2Cr_2O_7$) solution.	1a. Use a volumetric pipet.	
	2. Drain the 100.0 ml into a 1 liter volumetric flask.		
	3. Add distilled water up to the one liter mark on the flask.	3a. Use high quality distilled water with very low COD (See B.4).	
	4. Label the container.	4a. This is the 0.025 N potassium dichromate solution to be used for COD less than 50 mg/liter. 4b. Write the date and your name on the label.	
7. Ferroin indicator solution.	1. In a weighing boat, weigh 1.48 grams of ferroin, 1-10 (ortho) phenanthroline monohydrate ($\sigma C_{12}H_8N_2 \cdot H_2O$).	1a. You can purchase this indicator solution already prepared. 1b. You can use a balance with 0.01 gram sensitivity.	
	2. Put the weighed ferroin into a 250 ml beaker.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
7. Ferroin indicator solution (continued)	3. In a weighing boat, weigh 0.70 grams of ferrous sulfate with seven molecules of water of hydration.	3a. Use the same balance as above.	
	4. Put this into the same 250 ml beaker.		
	5. Measure 100 ml distilled water in a graduate.	5a. Use high quality distilled water with very low COD (See B.4).	
	6. Put the water into the 250 ml beaker containing the two weighed chemicals.		
	7. Stir to dissolve.	7a. Use a stirring rod or a magnet and magnetic stirrer apparatus. 7b. You can speed the dissolving process by heating the solution until it is just warm.	
	8. Put the indicator solution into dropper bottles.	8a. Use brown glass bottles. 8b. You need two bottles of about 50 ml capacity each.	
	9. Label the container.	9a. This is the ferroin indicator solution to be used in the test. Also write the date and your name on the label.	
8. 0.250 N Ferrous ammonium sulfate solution	1. In a weighing boat weigh out 98 grams of ferrous ammonium sulfate crystals. [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$].	1a. Use reagent grade ferrous ammonium sulfate. 1b. You can use a balance with 0.1 or 0.01 gram sensitivity. 1c. In this section, the letters FAS will be used when referring to this chemical.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
8. 0.250 N Ferrous ammonium sulfate solution (continued)	2. Put the weighed chemical into a 1 liter volumetric flask.		
	3. Fill the flask about two thirds full with distilled water.	3a. Use high quality distilled water with very low COD (See B.4.).	
	4. Swirl to dissolve the FAS.	4a. Support the bottom of the flask with your hand while swirling.	
	5. Measure 20 ml of concentrated sulfuric acid in a graduate.	5a. CAUTION: Sulfuric acid causes severe burns to the skin.	
	6. Tilt the 1 liter flask and slowly pour the acid down along the inside wall of the flask and into the solution.	6a. The solution may get slightly warm.	
	7. Swirl to mix the acid and the FAS solution.	7a. Support the bottom of the flask with your hand while swirling.	
	8. Add distilled water up to the one liter mark on the flask.	8a. Use high quality distilled water with very low COD (See B.4.).	
	9. Mix the solution by inverting the flask several times.		
	10. Pour the solution into a screw cap bottle.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
8. 0.250 N Ferrous ammonium sulfate solution (continued)	11. Label the container.	11a. This is 0.250 N ferrous ammonium sulfate solution. It is used for testing samples with COD greater than 50 mg/liter. 11b. It is unstable and should be stored in a dark bottle. 11c. When using it for tests, it must be standardized with potassium dichromate solution (Procedure C.). 11d. To use it for COD less than 50 mg/liter, you must dilute it to 0.025 N.	
9. 0.025 N Ferrous ammonium sulfate solution.	1. Measure 100.0 ml of 0.250 N ferrous ammonium sulfate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ solution.	1a. Use a volumetric pipet.	
	2. Drain the 100.0 ml into a 1 liter volumetric flask.		
	3. Add distilled water up to the one liter mark on the flask.	3a. Use high quality distilled water with very low COD (See B.4.).	
	4. Label the container.	4a. This is the 0.025 N ferrous ammonium sulfate solution to be used for COD less than 50 mg/liter. 4b. Write the date and your name on the label. 4c. The solution is unstable and should be stored in a dark bottle. 4d. When using it for tests, it must be standardized with potassium dichromate solution (Procedure C.).	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Ferrous Ammonium Sulfate Solution	1. Measure 15 ml distilled water.	1a. Use a graduate. 1b. Use high quality distilled water with very low COD.	
	2. Pour the water into a 250 ml Erlenmeyer flask.		
	3. Repeat steps 1 and 2 with a second flask for a duplicate test.		
	4. Prepare an ice bath.	4a. The depth of the water should be about one inch.	
	5. Place one flask into the ice bath.		
	6. Measure 10.0 ml of the 0.025 N potassium dichromate ($K_2Cr_2O_7$) solution.	6a. Use a volumetric pipet.	
	7. Drain the 10.0 ml into the 250 ml flask in the ice bath.		
	8. Swirl the beaker to mix the contents.		
	9. Let the flask in the ice bath.	9a. You want to cool the flask.	
	10. Repeat steps 5 through 9 for the duplicate test flask.		
	11. Measure 20 ml concentrated sulfuric acid (H_2SO_4).	11a. Use a graduate or an automatic dispenser checked for accurate delivery. 11b. CAUTION: Sulfuric acid causes severe burns to the skin.	V.C.11a. (p. 46)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Ferrous Ammonium Sulfate Solution (continued)	12. Tilt the 250 ml flask and slowly pour the acid down along the inside wall and into the solution.	12a. The solution and the flask will get warm.	
	13. Swirl the flask in the ice bath to mix the contents.	13a. You want to cool the flask to room temperature.	
	14. Remove the flask from the ice bath.	14a. The bottom of the flask may be slightly warm to the touch.	
	15. Repeat steps 11 through 14 for the duplicate test flask.		
	16. Put a buret clamp onto a titration stand.		
	17. Rinse and drain the inside of a clean 50 ml buret with about 15 ml of the ferrous ammonium sulfate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ solution which is about 0.025 N.	17a. In this section, the letters FAS will be used when referring to this ferrous ammonium sulfate solution. 17b. Put the FAS in a beaker so you can pour it into the buret.	
	18. Put the buret into the clamp on the stand.		
	19. Close the stopcock of the buret.		
	20. Add about 15 ml of FAS solution to the buret.	20a. Use a funnel.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Ferrous Ammonium Sulfate Solution (continued)	21. Check the tip of the buret for air bubbles.	21a. If there is an air pocket, swiftly turn the stopcock in a complete circle to expel it. You may have to repeat this turning of the stopcock.	
	22. You may have to add more FAS solution to the buret.	22a. You will need at least 10 ml of FAS for the titration.	
	23. Record the level of the solution in the buret.	23a. Use one of the columns on the sheet titled "Standardization of Ferrous Ammonium Sulfate (FAS) Solution." 23b. This number is "ml FAS at START of titration." 23c. Use the lowest part of the curve of the liquid (the meniscus) to take this reading. Some burets have a color stripe and you can see a colored point. Record the reading using the line where the point rests.	IX.Sheet II (p. 52) IX.C.23. (p. 52)
	24. Check that the 250 ml flask and contents are at room temperature before proceeding.	24a. You may have to put the flask back into the container of cold water to get this condition.	
	25. Add one drop of ferroin indicator to the mixture in the flask.	25a. The ferroin should be in a dropper bottle. If it isn't, use a medicine dropper to transfer it. 25b. One drop is used for a 45 ml mixture.	
	26. Gently swirl the flask to mix the contents.	26a. This ensures thorough mixing. 26b. Do not swirl any of the contents out of the flask. 26c. The mixture is a deep orange color.	
	27. Add about 8 ml of ferrous ammonium sulfate solution from the buret fairly rapidly while constantly swirling the mixture in the flask.	27a. You must constantly swirl the flask so the FAS solution comes into contact and reacts with the mixture in it.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Ferrous Ammonium Sulfate Solution (continued)	28. Now adjust the stopcock so the FAS solution in the buret goes into the flask more slowly and continue swirling the flask.	28a. The mixture in the flask gradually changes color during this titration. Beginning with a deep orange color, the mixture becomes green, then blue-green. At that stage, you are very close to the end point and the end point color of reddish-brown will appear at the surface of the mixture in the flask when drops of FAS reach it. When you observe this reddish-brown color, close the stopcock.	
	29. Now regulate the stopcock so the FAS solution in the buret goes into the flask one drop at a time.	29a. Swirl the flask after each drop is added. At the end point, one drop is enough to change the color of all of the solution to a reddish-brown.	
	30. Stop adding FAS when all the mixture in the flask is a reddish-brown color.	30a. This is the end point of the reaction in the flask.	
	31. Record the final level of the solution in the buret.	31a. Use the same column as before on the sheet titled "Standardization of Ferrous Ammonium Sulfate (FAS) Solution." 31b. This number is "ml FAS at END of titration."	IX.Sheet II (p. 52) IX.C.31. (p. 52)
	32. Repeat steps 22 through 31 for the duplicate test flask.		
	33. For each column of data, subtract the recorded "ml FAS at END of titration" and record the difference on your data sheet.	33a. Use the same columns on the sheet. 33b. This is the "ml of FAS solution used for the standardization" reaction.	IX.Sheet II (p. 52) IX.C.33. (p. 52)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Ferrous Ammonium Sulfate Solution (continued)	34. The differences found in step 33 above should agree within ±0.05 ml.	34a. If the differences do not agree within ±0.05 ml, repeat steps 1 through 34 (omitting 3, 10, 15, 32) to get a third difference which should agree with one of the differences recorded in step 33 within the ±0.05 ml limit.	
	35. Divide 0.250 by one of the "agreeing" ml differences found in step 34 above. Your answer should have four decimal places.	35a. Since the final answer is rounded off, you need not use averaged ml differences for this division. 35b. The division comes from using this formula: $\text{Normality}_{FAS} = \frac{\left(\text{ml potassium dichromate}\right)\left(\text{N potassium dichromate}\right)}{\text{ml ferrous ammonium sulfate}}$ or $N_{FAS} = \frac{(10.0)\ (0.025)}{\text{ml FAS}}$	
	36. Record this four decimal place answer.	36a. Use the same column on the sheet.	IX.C.36. (p. 52)
	37. Round off the answer to the division so the final answer has three decimal places.		
	38. Record this three decimal place answer.	38a. Use the same column on the sheet. 38b. This is the "Normality of the FAS solution." The number will be used later to calculate COD.	IX.C.38. (p. 52)
	39. Record the date.	39a. Use the same column on the sheet.	IX.C.39. (p. 52)
	40. Sign the sheet.	40a. Use the same column on the sheet.	IX.C.40. (p. 52)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Conditioning Flasks, Boiling Beads and Condensers.	1. If flasks, beads or condensers are new, if they were used for COD tests when the boiling mixture turned green, or if they were used for other tests, use these steps to condition them for use in COD tests.	1a. After conditioning, do not use this glassware for any other laboratory procedures. Even traces of organic materials on the glassware will react during the test and give higher results.	
	2. Measure 50 ml distilled water.	2a. Use a graduate. 2b. Use high quality distilled water with very low COD. (See B.4.).	
	3. Pour the water into the flask to be used in the test.	3a. Round bottom flasks can be supported by a heating mantle or a cork ring during these steps.	
	4. Repeat steps 2 and 3 for each flask to be used in the test.		
	5. Measure 25 ml 0.025 N potassium dichromate ($K_2Cr_2O_7$) solution.	5a. Use a graduate.	
	6. Pour this into one of the flasks.		
	7. Swirl the flask to mix the contents.		
	8. Repeat steps 5, 6, and 7 for each flask to be used in the test.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Conditioning Flasks, Boiling Beads and Condensers. (continued)	9. Measure 75 ml concentrated sulfuric acid (H_2SO_4).	9a. Use a graduate. 9b. CAUTION: Sulfuric acid causes severe burns to the skin.	
	10. Tilt the flask and slowly pour the acid down the inside wall.	10a. The solution and flask get very hot.	
	11. Swirl the flask to mix the contents.		
	12. Repeat steps 9, 10 and 11 for each flask to be used in the test.		
	13. Add 5 glass beads to each flask containing mixtures.		
	14. Carefully swirl each flask again.	14a. CAUTION: You must thoroughly mix the contents of the flask to avoid an explosion during procedure.	
	15. Check the heat of each flask.	15a. If the flasks are just warm to the touch, go to the next step. If the flasks are very hot, put them one by one down into a container of cold water to get rid of excess heat.	
	16. Use a paper towel to wipe off any water droplets on the outside of the flask.		
	17. Attach one of the flasks to a condenser.	17a. The condenser is described in the equipment list, page 6.	
	18. Gently twist the flask while gently pushing it upward onto the condenser.	18a. This ensures a good seal.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Conditioning Flasks, Boiling Beads and Condensers (Continued)	19. Center the flask on/in a heating surface.	19a. Options for heaters are described in the equipment list, page 6.	
	20. Repeat steps 17, 18 and 19 for each flask you are conditioning.		
	21. Do not turn on the water to cool the condensers.	21a. You want the vapors of this cleaning mixture to move all the way up inside the condenser.	
	22. Turn on the heat source for each flask.		
	23. When the contents of the flasks begin to boil, keep looking to see if vapors come out of the top of the condenser.		
	24. Note the time when you see vapors coming out.		
	25. Let the boiling continue 5 to 10 minutes.		
	26. Turn off the heat source for each flask.		
	27. Allow flasks to cool.	27a. This takes 10 to 15 minutes.	
	28. Squirt distilled water into the opening at the top of each of the condensers.	28a. Use up to 25 ml of high quality distilled water with very low COD. 28b. This rinses any condensates down the inside walls and into the flask.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Conditioning Flasks, Boiling Beads and Condensers (Continued)	29. Lightly plug the top opening of each condenser with clean glass wool.	29a. This prevents contamination from air-borne particles. The plug can be left in the condenser during the test.	
	30. Using a twisting motion, partially disconnect one of the flasks from the condenser.		
	31. Squirt distilled water over the condenser tip, allowing this rinsing to go down into the flask.	31a Do not touch the condenser tip with your fingers, paper towels, etc. Organic contamination of the tip could result.	
	32. Remove the flask from under the condenser.		
	33. Squirt distilled water on the inside of the neck of the flask, allowing this rinsing to go down into the flask.		
	34. Turn on the cold water in a sink.	34a. You will have to dispose of the cleaning mixture. 34b. Plumbing must be able to tolerate acid.	
	35. Slowly pour the contents of the flask directly into the drain.	35a. The glass beads should stay in the flask. 35b. You could pour the contents through a Buchner funnel to catch the glass beads.	
	36. Let the cold water run at least 5 minutes.	36a. This dilutes the acid in the drain.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Conditioning Flasks, Boiling Beads and Condensers (Continued)	37. Use tap water to rinse the flask 3 times.	37a. The glass beads should stay in the flask for these rinsings or rinse those in the Buchner funnel.	
	38. Use distilled water to rinse the flask 3 times.	38a. Use high quality distilled water with very low COD. 38b. The glass beads should stay in the flask for these rinsings or rinse those in the Buchner funnel.	
	39. Drain the last of the distilled water from the flask.	39a. The glass beads stay in the flask. If you have used a Buchner funnel, roll the beads back into the flask.	
	40. Attach the flask to the rinsed condenser.	40a. The flask should stay there until it is used for a test.	
	41. Repeat steps 30 through 40 for each flask that is being prepared for use.		
E. Conditioning Glassware Other Than Flasks, Boiling Beads or Condensers.	1. If the glassware is new, if it has been used to measure COD samples, or if it has been used for tests other than COD, use these steps to condition it for use.	1a. Glassware is included in the equipment list on pages 6, 7 and 8. 1b. This section applies to glassware used to prepare and store reagents as well as to glassware used during the COD test. 1c. After conditioning, do not use this glassware for other laboratory procedures. Even traces of organic materials on the glassware will react during the test and give higher results.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Conditioning Glassware Other Than Flasks, Boiling Beads or Condensers. (Continued)	2. Measure 250 ml distilled water.	2a. Use a graduate. 2b. Use high quality distilled water with very low COD. (See B.4.).	
	3. Pour the water into a clean bottle.	3a. The bottle will be used for storage so have one with a screw cap. You can use a clean acid bottle.	
	4. Measure 125 ml of 0.025 N potassium dichromate solution.	4a. Use a graduate.	
	5. Pour the measured potassium dichromate ($K_2Cr_2O_7$) into the same bottle.		
	6. Put the bottle into an ice bath.	6a. The depth of ice water should be an inch above the level of acid in the bottle.	
	7. Keeping the bottle in the ice water, swirl the contents in the bottle.	7a. You want to mix it.	
	8. Leave the bottle in the ice bath.	8a. You want it to get cool.	
	9. Measure 375 ml of concentrated sulfuric acid (H_2SO_4).	9a. Use a graduate. 9b. Use reagent grade acid. 9c. CAUTION: Sulfuric acid causes severe burns to the skin.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Conditioning Glassware Other Than Flasks, Boiling Beads or Condensers. (Continued)	10. Tilt the bottle in the ice bath and slowly pour the acid down the inside wall.	10a. The solution and bottle get hot.	
	11. Keeping the bottle in the ice bath, swirl the contents in the bottle.	11a. You want to mix it.	
	12. Check that the bottle is cool enough to handle.		
	13. Label the bottle.	13a. This is Conditioning Solution for COD glassware. Also mark the date and your name.	
	14. Use the warm solution to rinse over the walls of the glassware.	14a. You can store the solution for future use. In this case, pour an adequate volume into a beaker and warm the solution on a hot plate. 14b. CAUTION: The sulfuric acid in the solution causes severe burns to the skin.	
	15. Discard the used solution.	15a. Turn the cold water tap on in a sink. 15b. Slowly pour the acid down the drain. 15c. Let the tap run at least 5 minutes.	
	16. Repeat steps 14 and 15 two more times for each piece of glassware to be conditioned.		
	17. Rinse each piece of glassware with tap water 3 times.	17a. CAUTION: The first rinse contains a significant amount of sulfuric acid.	
	18. Rinse each piece of glassware with distilled water 3 times.	18a. Use high quality distilled water with low COD.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Conditioning Glassware Other Than Flasks, Boiling Beads or Condensers. (Continued)	19. Let the glassware drain dry.		
	20. Store the glassware separately from glassware used for tests other than COD.		
	21. Store unused solution for future use.		
F. Oxidation of the Sample and Blank	1. Remove two reflux flasks from COD flask-condenser assemblies.	1a. Equipment is described on pages 6, 7 and 8. 1b. Each flask should have 5 glass beads in it. 1c. All flasks, glass beads and condensers should have been used previously for COD tests. If any flask, beads or condenser is new or has been used for other tests, each must be conditioned according to Procedure D. in this EMP.	
	2. Mark the sample identification on the outside of one of the flasks.	2a. Use a wax marking pencil. 2b. See the label on the sample bottle for an identification code. 2c. In this procedure we will call this the Sample flask.	
	3. Mark the word, "Blank" on the outside of the second flask.	3a. You will prepare a blank and test it in the same manner as the sample. 3b. In this procedure, we will call this the Blank flask.	VII.F.3a. (p. 48)
	4. Measure 1 gram of mercuric sulfate ($HgSO_4$).	4a. Use a 1 gram reagent spoon.	
	5. Place the mercuric sulfate in the Sample flask.	5a. Round bottom flasks can be supported by a heating mantle or a cork ring during these steps.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Oxidation of the Sample and Blank (continued)	6. Repeat steps 4 and 5 for the Blank flask.		
	7. Shake the bottle of sample.		
	8. Draw 50.0 ml of sample into a pipet.	8a. Use a clean volumetric pipet. 8b. Use a pipet bulb. 8c. For samples that turn green during the test and which cannot be titrated to an acceptable end point, you may need to dilute the sample to a final volume of 50.0 ml at this step. You won't know you have to do this until you have run 50.0 ml of the sample through the test up to H. Quantification, Step 12. To avoid this uncertainty, you can prepare dilutions now. See Training Guide.	VII.F.8c. (p. 49)
	9. Deliver the 50.0 ml into the Sample flask.	9a. Record "S, ml Sample Used" on the "Typical Laboratory Data Sheet" in this EMP.	IX.F.9. (p. 51)
	10. To prepare the blank, draw 50.0 ml of distilled water into another pipet.	10a. Use high quality distilled water with very low COD. 10b. Use a clean volumetric pipet. 10c. Use a pipet bulb.	
	11. Deliver the 50.0 ml distilled water into the Blank flask.		
	12. Draw 5.0 ml concentrated sulfuric acid (H_2SO_4) into a pipet.	12a. CAUTION: Sulfuric acid causes severe skin burns. 12b. Use a clean 10 ml graduated pipet and a pipet bulb, or else an automatic dispenser checked for 5.0 ml delivery.	V.F.12b. (p. 46)
	13. Deliver the 5.0 ml of acid into the Sample flask.	13a. Tilt the flask and deliver the acid down along the inside wall.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Oxidation of the Sample and Blank (continued)	14. Rinse off the outside of the pipet at a sink.	14a. Use tap water to rinse any acid into the sink. 14b. Let the water continue to run for a few minutes.	
	15. Swirl the contents of the flask.	15a. Most of the mercuric sulfate dissolves.	
	16. Repeat steps 12 through 15 to add 5.0 ml of acid to the Blank flask and rinse the pipet.		
	17. Prepare an ice bath.	17a. The depth of the water should be about one inch.	
	18. Place the Sample flask into the ice bath.		
	19. Draw 25.0 ml of 0.025 N potassium dichromate ($K_2Cr_2O_7$) solution into a pipet.	19a. Use a clean volumetric pipet. 19b. Use a pipet bulb.	
	20. Swirl the flask as you slowly add the 25.0 ml of 0.025 N potassium dichromate solution.		
	21. Let the flask in the ice bath.	21a. You want to cool the flask.	
	22. Measure 70 ml of sulfuric acid-silver sulfate (H_2SO_4-Ag_2SO_4) solution.	22a. CAUTION: Sulfuric acid causes severe skin burns. 22b. Use a clean 100 ml graduate or use an automatic dispenser checked for accurate delivery.	V.F.22b. (p. 46)
	23. Tilt the flask in the ice bath and swirl to continuously mix as you slowly add the acid-silver sulfate solution down the inside wall of the flask.	23a. If the acid-sulfate solution is added too rapidly, heat at the surface of the solution can cause spattering upward.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Oxidation of the Sample and Blank (continued)	24. Swirl the flask in the ice bath.	24a. You want to cool the flask.	
	25. Remove the flask from the ice bath.	25a. The bottom part of the flask may still be warm.	
	26. Wipe the water off the outside of the flask.	26a. Use a paper towel.	
	27. Carefully swirl the flask again.	27a. CAUTION: You must thoroughly mix the contents of the flask to avoid an explosion during reflux. 27b. CAUTION: Do not swirl so vigorously that the contents come out of the flask.	
	28. Repeat steps 18 through 27 to add potassium dichromate and sulfuric acid-silver sulfate solutions to the Blank flask.		
	29. Attach the Sample flask to a condenser.	29a. The condenser is described in the equipment list, page 6.	
	30. Gently twist the flask while gently pushing it upward onto the condenser.	30a. This ensures a good seal.	
	31. Center the flask on/in a heating surface.	31a. Choices for heaters are described in the equipment list, page 6.	
	32. Repeat steps 29 through 31 to attach the Blank flask to a condenser.		
	33. Start the circulation of cooling water through the two condensers.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Oxidation of the Sample and Blank (continued)	34. Turn on the heat source for each flask.		
	35. When the contents of the flasks begin to boil, record the date and time.	35a. Use the "Typical Laboratory Data Sheet." 35b. Use the columns for the sample(s) and blank.	IX.F35. (p. 51)
	36. Regulate the heat sources if necessary.	36a. Adjust the heat to maintain a gently rolling boil in each flask.	
	37. Reflux the contents of the flasks for two hours.	37a. The mixtures in the flasks are usually a dark orange color during this period. If some turn to a green color, the potassium dichromate may be completely reacted. Continue the test for such flasks, though, because there may be enough potassium dichromate left to titrate later on in Procedure H. Quantitation.	
		37b. If the samples are known to require less time for complete oxidation, less reflux time is acceptable.	VII.F37b. (p. 50)
G. Rinsing and Removing Flasks from Condensers (continued)	1. Turn off the heat under the flask-condenser assemblies.	1a. The contents of the flasks should have gently boiled for 2 hours.	
	2. Allow the flasks to cool.	2a. This takes 10 to 15 minutes. 2b. Placing an evaporating dish upside down between the flask and the heating surface makes for faster cooling.	
	3. Squirt distilled water into the opening at the top of the condenser which is attached to the flask containing the sample.	3a. Use high quality distilled water with very low COD. 3b. You want to rinse any condensates down the inside walls and into the flask. 3c. Use up to 25 ml of water.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Rinsing and Removing Flasks from Condensers (continued)	4. Using a twisting motion, partially disconnect the flask from the condenser.	4a. Point the lower tip of the condenser down into the flask. 4b. Be very careful to avoid adding organic contimination to the joint and into the inside of the neck of the flask. Do not touch these parts with your fingers, paper towels, etc.	
	5. Squirt distilled water over the condenser tip, allowing this rinsing to go down into the flask.		
	6. Remove the flask from under the condenser.		
	7. Squirt distilled water on the inside of the neck of the flask, allowing this rinsing to go down into the flask.	7a. You want to rinse any condensates down into the flask.	
	8. Squirt distilled water down along the inside of the walls of the flask.	8a. If a 300 ml/round bottom flask has been used, transfer the mixture to a 500 ml Erlenmeyer flask. Squirt distilled water down the inside walls of the original flask and pour the rinsing into the Erlenmeyer flask. Repeat this rinsing of the original flask three times.	
	9. Add enough distilled water to the flask containing the sample to bring the final volume to about 300 ml.	9a. If volumes are marked on the flask, add distilled water to the 300 ml mark. 9b. If volumes are not marked on the flask, estimate the amount of water needed to bring the volume to 300 ml, measure it in a graduate and add it to the flask. (The original mixture totaled 150 ml and rinsings of the condenser, joint and flask would range from 40 to 70 ml.)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Rinsing and Removing Flasks from Condensers (continued)	10. Put this flask near the titration stand.		
	11. Squirt distilled water into the opening at the top of the condenser which is attached to the flask containing the distilled water blank.	11a. Use high quality distilled water with very low COD. 11b. You want to rinse any condensates down the inside walls and into the flask. 11c. Use up to 25 ml of water.	
	12. Repeat steps 4 through 10 above to rinse inner walls of this condenser and flask and to bring the final volume to about 300 ml.		
H. Quantification: Titration of Sample and Blank	1. Rinse and drain the inside of a clean, 50 ml buret with about 15 ml of ferrous ammonium sulfate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ solution of known normality.	1a. Ferrous ammonium sulfate solution is unstable and must be standardized on the day you use it so the normality is known. The procedure to do this is described in "C. Standardization of Ferrous Ammonium Sulfate Solution."	
	2. Put the buret into the clamp on the titration stand.		
	3. Close the stopcock of the buret.		
	4. Add about 15 ml of the ferrous ammonium sulfate solution.	4a. Use a funnel. 4b. In this section, the letters FAS will be used when referring to the ferrous ammonium sulfate solution of known normality.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Quantification: Titration of Sample and Blank (continued)	5. Check the tip of the buret for air bubbles.	5a. If there is an air pocket, swiftly turn the stopcock in a complete circle to expel it. You may have to repeat this turning of the stopcock.	
	6. Add more FAS solution to the buret.	6a. You will need up to 25 ml of FAS for each sample and blank.	
	7. Record the level of the solution in the buret.	7a. On the "Typical Laboratory Data Sheet" in the column with the sample identification information. 7b. This is the "ml FAS at START of titration".	IX.Sheet I. (p. 51) IX.H.7. (p. 51)
	8. Check that the flask containing the sample is at room temperature before proceeding.	8a. You may have to put the flask into a pan of cool water to get this condition.	
	9. Gently swirl the contents of the flask containing the sample.	9a. This ensures thorough mixing. 9b. Do not swirl any of the contents out of the flask.	
	10. Add 10 drops of ferroin indicator solution to the mixture in the flask.	10a. The ferroin should be in a dropper bottle. If it isn't, use a medicine dropper to transfer it. 10b. Ten drops are used for a 300 ml mixture.	
	11. Again, gently swirl the contents of the flask.	11a. This ensures thorough mixing. 11b. The mixture is a deep orange color.	
	12. Add FAS solution from the buret fairly rapidly, while constantly swirling the mixture in the flask.	12a. You must constantly swirl the receiving flask so that the FAS solution comes into contact and reacts with all the mixture in it. 12b. The mixture in the flask will gradually change color becoming green, then blue-green. When the addition of FAS solution makes a reddish-brown color at the surface of the sample solution, close the stopcock.	

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Quantification: Titration of Sample and Blank (continued)		12c. If a sample had turned a green color during the 2-hour boiling period, there may be no potassium dichromate solution left in the flask. If you add up to 22 ml of FAS solution to such a mixture and cannot observe the color changes described above in 12b., stop the titration. You should do the test over, using a smaller volume of sample. (See Training Guide). Also, the flask, boiling beads and condenser used for that sample will have to be conditioned before re-use. (See Procedure D.)	VII.H.12c. (p. 49)
	13. Now adjust the stopcock so the FAS solution in the buret goes into the flask one drop at a time.	13a. Swirl the flask after each drop is added. At the end point, one drop is enough to change the color of all the solution to a reddish brown.	
	14. Stop adding FAS solution when all the mixture in the flask is a reddish brown color.	14a. This is the end point of the reaction in the flask.	
	15. Record the final level of the FAS solution in the buret.	15a. On the data sheet, this is "ml FAS at END of titration". 15b. Use the column for this sample.	IX.H.15 (p. 51)
	16. Subtract the recorded "ml of FAS at beginning of titration "from" ml of FAS at end of titration" and record the difference on your data sheet.	16a. On the data sheet, this is "B, ml. FAS used to titrate the Sample". 16b. Use the column for this sample.	IX.H.16. (p. 51)
	17. To titrate the blank, first check the level of the FAS solution in the buret.	17a. You will need up to 25 ml of FAS solution to titrate the blank. Add more FAS if necessary.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Quantification: Titration of Sample and Blank (continued)	18. Record the level of the FAS solution in the buret.	18a. On line one of a column on the data sheet, write "Blank" as the identification. 18b. In that column, record the level of FAS as "ml FAS at START of titration".	IX.Sheet I (p. 51) IX.H.18. (p.51)
	19. Check that the flask containing the blank is at room temperature before proceeding.	19a. You may have to put the flask into a pan of cool water to get this condition.	
	20. Gently swirl the contents of the flask containing the distilled water blank.	20a. This ensures thorough mixing. 20b. Do not swirl any of the contents out of the flask.	
	21. Repeat steps 10 through 14 above to add the FAS solution from the buret until all the mixture in the flask is reddish brown.		
	22. Record the final level of the FAS solution in the buret.	22a. On the data sheet, this is "ml FAS at END of titration." 22b. Use the column for the blank.	IX.H.22. (p. 51)
	23. Subtract the recorded "ml of FAS at beginning of titration" from "ml of FAS at end of titration" and record the difference on your data sheet.	23a. On the data sheet, this is "A, ml FAS used to titrate the Blank". 23b. Use the column for the blank.	IX.H.23. (p. 51)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Clean Up	1. Carefully pour the contents of both the Sample and the Blank flasks through a cleaned Buchner funnel and into a storage container.	1a. The glass beads should stay in the funnel. 1b. The storage container should be glass or thick plastic with a screw cap. 1c. These mixtures have up to 25% concentrated sulfuric acid so handle and store them with caution. 1d. These mixtures also contain mercury complexes which need special treatment for disposal.	VI.I.1d. (p. 47)
	2. Use tap water to rinse each flask 3 times.	2a. If the flask contained a mixture which turned green during the 2 hour boiling period, the flask, beads and the condenser will have to be conditioned before re-use for a COD test. (See Procedure D.)	
	3. Use distilled water to rinse each flask 3 times.	3a. Use high quality distilled water with very low COD (See B.4.)	
	4. Wipe any wax markings off the outside of the flasks.		
	5. Use tap water to rinse the beads 3 times.	5a. The beads are held in the Buchner funnel.	
	6. Use distilled water to rinse the beads 3 times.	6a. The beads are still in the funnel.	
	7. Transfer 5 glass beads to each rinsed reflux flask.	7a. Do not contaminate the beads at this step. Use a spatula to roll the beads from the edge of the funnel to the flask or use forceps to make the transfer.	
	8. Attach each flask to a condenser used and rinsed during the test.	8a. The flasks should stay there until they are used again.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Clean Up (continued)	9. Drain any ferrous ammonium sulfate solution out of the buret.	9a. This can be put directly down the drain of a sink.	
	10. Use tap water to rinse the inside of the buret 3 times.		
	11. Use high quality distilled water to rinse the buret 3 times.		
	12. Put the buret back in the titration stand but upside down with the stopcock open.	12a. The buret can drain completely. 12b. This buret should be used only for the COD test. Even traces of organic materials from other solutions may result in errors in future COD titrations.	
	13. Other glassware (pipets, etc.) used during the test should be rinsed with tap water.		
	14. As soon as possible, this other glassware should be cleaned using Procedure E.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Calculations	1. Use the following steps to calculate COD, mg/liter:	1a. The calculation formula is: Chemical Oxygen Demand, mg liter = $\frac{(A-B)\ N \times 8000}{S}$ Where: A= ml FAS to titrate the Blank B= ml FAS to titrate the Sample N= normality of FAS S= ml Sample Used 8000 converts to COD, mg/liter	
		1b. The "Typical Laboratory Data Sheet" has the steps and an example for doing this calculation.	IX.Sheet I (p. 51)
		1c. Numbers used in the examples below are from the example in the last columns on the "Typical Laboratory Data Sheet".	IX.Sheet I (p. 51)
	2. Subtract "B, ml FAS used to titrate the Sample" on line 10 from "A, ml FAS used to titrate the Blank" on line 9.	2a. Example on data sheet: line 9 : 23.55 line 10: 15.00 Difference= 8.55	
	3. Write the difference on line 11 of the data sheet.	3a. This has been done for the example on the data sheet.	IX.J.3. (p. 51)
	4. Record "N, normality of FAS" on line 12 of the data sheet.	4a. This number is calculated as shown by the example on the sheet titled "Standardization of Ferrous Ammonium Sulfate (FAS) Solution."	
		4b. The example number, 0.024, from C.38 on that sheet has been recorded on line 12 of the data sheet.	IX.J.4. (p. 51)

EFFLUENT MONITORING PROCEDURE: Determination of Chemical Oxygen Demand

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Calculations (continued)	5. Multiply the difference on line 11 by "N, normality of FAS" on line 12.	5a. Example on data sheet: $8.55 \times 0.024 = 0.2052$	
	6. Record the product on line 13.	6a. This has been done for the example on the data sheet.	IX.J.6. (p. 51)
	7. Divide 8000 by "S, ml Sample Used" which is recorded on line 6.	7a. Example on data sheet: $\frac{8000}{50.0} = 160$	
	8. Record the answer on line 14.	8a. This has been done for the example on the data sheet.	IX.J.8. (p. 51)
	9. Multiply line 13 by line 14.	9a. Example on data sheet: $0.2052 \times 160 = 32.8320$	
	10. Record the product on line 15.	10a. This has been done for the example on the data sheet.	IX.J.10. (p. 51)
	11. Round off the number on line 15 to the nearest whole number of mg/liter.	11a. 32.8320 becomes 33. 11b. If your answer for a sample is greater than 50 mg/liter, you should start using the 0.250 N solutions of potassium dichromate and ferrous ammonium sulfate for samples from that same source. See the Training Guide.	I.J.11b. (p. 44)
	12. Record this number on line 16.	12a. This has been done for the example on the data sheet.	IX.J.12 (p. 51)
	13. Sign the data sheet on line 17.	13a. This has been done for the example on the data sheet.	IX.J.13. (p. 51)

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts-Mathematics
III	Educational Concepts-Science
IV	Educational Concepts-Communications
V*	Field & Laboratory Equipment
VI*	Field & Laboratory Reagents
VII*	Field & Laboratory Analysis
VIII	Safety
IX*	Records & Reports

Training guide materials are presented here under the heading marked. These standardized headings are used throughout this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	The Chemical Oxygen Demand (COD) Test provides an estimate of the proportion of sample matter susceptible to oxidation by rigorous oxidation conditions. Some inorganic compounds may be oxidized but most of the reaction involves organic compounds. Thus the COD Test provides a commonly used estimate of organic materials in water samples.	
J.11b.	The procedure described in this EMP is for the range of 5 to 50 mg/liter COD one expects in treatment plant effluents. If you use this EMP procedure and get results greater than 50 mg/liter COD, you should do the test in the same manner as described in the EMP but use more concentrated solutions (0.250 N instead of 0.025 N) of potassium dichromate and of ferrous ammonium sulfate. For "B. Reagent Preparation", you would not make number 6 (0.025 N potassium dichromate solution) nor number 9 (0.025 N ferrous ammonium sulfate solution). Any place in the procedure that refers to 0.025 N concentrations of either of these solutions should be read as 0.250 N. All other instructions and information are to be followed as written.	
	The Test described in this instruction can be found in the 1974 EPA Methods Manual on Page 21, entitled Chemical Oxygen Demand (Low Level). Other references which have acceptable procedures for this test for NPDES purposes are: 14th ed. Standard Methods on page 550 and 1975 ASTM Part 31 on page 472.	Methods for Chemical Analysis of Water and Wastes, 1974, EPA, MDQARL Cincinnati, Ohio 45268 p. 21. Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, N.Y. p. 550 Annual Book of Standards, Part 31, Water, 1975, ASTM, Philadelphia, PA, p. 472

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B2.1d. B3.5b. C.11a. F.12b. F.22b.	AUTOMATIC DISPENSERS: Since sulfuric acid causes severe burns to the skin, you may choose to use a glass, automatic dispenser (pipet) to store and measure the two reagents involving this acid. Use the manufacturer's instructions to fill and prime the dispenser and to make the initial setting of the delivery volume. Sulfuric acid is heavier than water so this delivery volume must be checked. Do this by delivering the acid into a clean, dry graduate. Allow the acid to "settle" in the graduate, then read the volume. If that volume is more or less than it should be (see below), adjust the delivery setting on the dispenser accordingly. Then check the new setting for accurate delivery, using another clean, dry graduate. Continue this procedure until you are satisfied that the delivery volume is accurate. The final volumes required for the concentrated sulfuric acid reagent are 5 ml and 20 ml so adjust a dispenser to deliver 5 ml. Then dispense the 5 ml four times for the 20 ml requirement. The final volume required for the sulfuric acid - silver sulfate reagent is 70 ml. In this case, adjust a dispenser to deliver 10 ml. Then dispense the 10 ml seven times for the 70 ml requirement.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
I.1d.	DISPOSAL OF MERCURY-CONTAINING WASTES: These wastes can be treated to convert the soluble mercury complexes to some insoluble formation. Some refiners of mercury are willing to accept shipments of such precipitates to recycle the metal. Ask your City, County, or State Pollution Control Agency for specific instructions on how you are to dispose of COD test wastes.	Dean, Williams, Wise: "Disposal of Mercury Wastes from Water Laboratories," Environmental Science and Technology Vol. 5, No. 10, 1971. p. 1044 Maag and Hecker: "Recovery of Mercury in Solution," Journal of Environmental Quality, Vol. 1, No. 2, 1972, p.192.

BLANKS:

B4.1h. F.3a. You run a blank by using distilled water instead of sample water and testing that distilled water in the same way you test samples. By doing this, you are checking the COD of the distilled water and of the reagents used in the test. You use the titration results for the blank in the calculation formula to correct your COD values for samples.

Some contamination is expected to show up and affect the titration results. However, if the blank requires less than 90% of the ml of ferrous ammonium sulfate solution that would be required to titrate a situation of "no contamination," it is a signal to you that the distilled water or reagents are contributing contamination and should be checked.

EXAMPLE - If 25.0 ml of 0.025 N potassium dichromate solution are used to try to oxidize a blank containing no oxidizable contamination, it would take 25.0 ml of 0.025 N ferrous ammonium sulfate solution to react with the remaining potassium dichromate during the Quantitation Titration Procedure. 90% of 25.0 ml of FAS would be 22.5 ml. If you use less than 22.5 ml of FAS for two or more blanks, a check of the distilled water and of the reagents is advisable.

See "B. Reagent Preparation, Procedure 4. Distilled Water" for information about checking the quality of distilled water and about storing it.

To check reagents, consult your laboratory records to see which reagent was made most recently. Using the "E. Conditioning..." Procedure, clean the glassware required and then prepare a fresh supply of that reagent. Use the fresh reagent and run a blank. If the blank results are still too high, you should purchase a new supply of the chemical to make your reagent solution. If you always purchase reagent grade chemicals and take care not to contaminate them with dirty spatulas, etc., you should not have problems with them.

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

F.8c.
H.12c.

SMALLER VOLUMES OF SAMPLE FOR THE TEST:
If you cannot titrate a 50.0 ml sample to an acceptable end point during "H. Quantitation step 12," you will have to re-run the test beginning with "F. Oxidation of the Sample and Blank". At F. Step 8, you will have to use less sample and then add distilled water to make the 50.0 ml volume. The workable proportions can only be determined by trial and error. You might prepare two mixtures at this step such as:
Add 10.0 ml sample, then 40 ml distilled water to the flask
Add 25.0 ml sample, then 25 ml distilled water to the flask.

(1) Use a pipet to measure the sample,
(2) Use a graduate to measure the water,
(3) Use high quality distilled water with low COD.
(4) If you get results for both dilutions, use the results for the 25.0 ml sample.
(5) For future tests of samples from the same source, at step F8 use the dilution proportions you found workable.
(6) Do not change the volumes of any other solutions in the test. The volumes as given in the EMP are critical conditions of the test.
(7) If you cannot titrate as low as 10.0 ml of sample (with 40 ml water added to the test mixture) during "H. Quantitation, step 12", you will have to do the test using the 10.0 ml sample and more concentrated potassium dichromate and ferrous ammonium sulfate solutions (the 0.250 N solutions are used). See section I in the Training Guide for a discussion of this.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.37b.	TWO HOUR OXIDATION (BOILING) PERIOD: Some samples contain materials that can be oxidized in the COD test conditions within a very short time period. If your samples always have the same materials in them, you may want to check them and see if you might use a shorter oxidation (boiling) period. Prepare two test mixtures from the same sample. Boil one for two hours, boil the other for say 30 minutes. Complete the test as usual and calculate COD results for each. Do 6 other such duplicate tests on 6 other samples. These should be done over a period of time on samples collected from the same source over a period of time. Compare the results from the seven tested by using the usual 2 hours with the results from the seven tested by using a shorter oxidation (boiling) period. If the results are the same or if they agree within ± 4 mg/liter COD, you may use the shorter oxidation (boiling) time for future samples from the same source. About once every 10 times you perform the test on such samples you should check that they continue to be the same composition. Do this by preparing a duplicate test mixture, using a two hour boiling period for the second mixture, and comparing the results for agreement as above.	Methods for Chemical Analysis of Water and Wastes, 1974 EPA, MDQARL Cincinnati, OH 45268, p. 23. Handbook for Analytical Quality Control in Water and Wastewater Laboratories. 1971, EPA, MDQARL, Cincinnati, Ohio 45268 p. 6-1.

IX.SHEET I

Typical Laboratory Data Sheet for Chemical Oxygen Demand, mg/liter

Name of Plant ______________________________

A.5	Identification				Blank	EFF #1	1
A.5	Type (grab, composite)					Composite	2
A.5	Date and Time Collected					3/17/75 0600-1200	3
A.5	Sample Collector					Tom Sampler	4
F.35	Date and Time Boiling Began					3/17/75 1300	5
F.9	RECORD: S, ml Sample Used					50.0	6
H.15 H.22	ml FAS * at END of titration				38.55	20.00	7
H.7 H.18	ml FAS * at START of titration				15.00	5.00	8
H.23	A, ml FAS* used to titrate the Blank				23.55	23.55	9
H.16	B, ml FAS * used to titrate the Sample					15.00	10
J.3	SUBTRACT B (line 10) from A (line (9)					8.55	11
J.4	RECORD: N, normality of FAS * (Calculated on Standardization Sheet,C.29)					0.024	12
J.6	MULTIPLY ml Difference of FAS * (line 11) by Normality of FAS (line 12)					0.2052	13
J.8	DIVIDE 8000 by S, ml Sample Used (See line 6)					160	14
J.10	MULTIPLY line 13 by line 14					32.8320	15
J.12	ROUND OFF line 15 to the nearest whole number of mg/liter					33	16
J.13	Signature					Jim Analyst	17

* FAS means Ferrous Ammonium Sulfate Solution

CALCULATION FORMULA: $\text{COD, mg/liter} = \frac{(A-B)N \times 8000}{S}$

STANDARDIZATION OF FERROUS AMMONIUM SULFATE (FAS) SOLUTION IX SHEET II

				Flask 1	Duplicate	
C.31	ml FAS at END of titration			24.60	35.15	1
C.23	ml FAS at START of titration			14.00	24.60	2
C.33	ml FAS used for Standardization (SUBTRACT ml FAS at START on line 2 from ml FAS at END on line 1)			10.60	10.55	3
C.36	DIVIDE 0.250* by the ml difference on line 3 to a 4 decimal place answer.				0.0236	4
C.38	Normality of the FAS solution (ROUND OFF line 4 to 3 decimal places)				0.024	5
C.39	Date				3/17/75	6
C.40	Signature				Jim Analyst	7

* From the formula:

$$\text{Normality FAS} = \frac{(10.0\ \text{ml potassium dichromate})\ (0.025\ \text{N potassium dichromate})}{\text{ml FAS}}$$

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF TOTAL KJELDAHL NITROGEN

as applied in

WASTEWATER TREATMENT FACILITIES
AND IN THE
MONITORING OF EFFLUENT WASTEWATERS

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

This Operational Procedure was developed by:

NAME William T. Engel

ADDRESS Charles County Community College
P. O. Box 910
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POSITION Assistant Professor of Chemistry

EDUCATION & TECHNICAL BACKGROUND

BS - Saint Francis College, Loretto, Pennsylvania

MS - Xavier University, Cincinnati, Ohio

6 years Instructor;

Instructor - Associate Professor (Chemistry)

EFFLUENT MONITORING PROCEDURE: Determination of Total Kjeldahl Nitrogen

1. Objective:

   To determine the Total Kjeldahl Nitrogen content of an effluent.

2. Description of Analysis:

   The procedure converts nitrogen components of biological origin such as amino acids, proteins, and peptides to ammonia. Two alternatives are listed for the determination of ammonia after distillation: the titrimetric method which is applied to concentrations above 1 mg N/liter and the colorimetric method which is applicable to concentrations below 1 mg N/liter.

3. Applicability of this Procedure:

   a. Range of Concentration:

      Colorimetric Method - 0.03 to 1.0 mg NH_3-N/liter

      Titrimetric Method - 1.0 to 25 mg NH_3-N/liter

      (The range of these methods may be extended for samples by dilution.)

      NOTE: A range from 0.05 to 1400 mg NH_3-N/liter is available by using an ammonia selective ion electrode. A separate EMP on this method is available.

   b. Pretreatment of Samples:

      The Federal Register Guidelines do not specify any pretreatment.

   c. Treatment of Interferences in Samples:

      The Source of Procedure* does not note any interferences to this determination.

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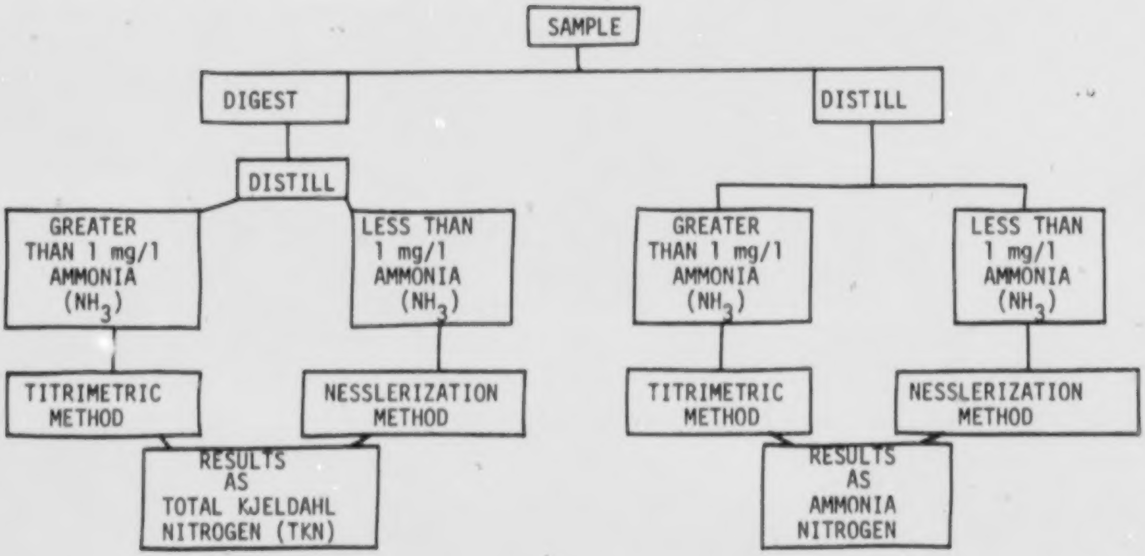
*Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, Ohio. page 175.

FLOW CHART


SAMPLE
DIGEST
DISTILL
DISTILL
GREATER THAN 1 mg/l AMMONIA (NH_3)
LESS THAN 1 mg/l AMMONIA (NH_3)
GREATER THAN 1 mg/l AMMONIA (NH_3)
LESS THAN 1 mg/l AMMONIA (NH_3)
TITRIMETRIC METHOD
NESSLERIZATION METHOD
TITRIMETRIC METHOD
NESSLERIZATION METHOD
RESULTS AS TOTAL KJELDAHL NITROGEN (TKN)
RESULTS AS AMMONIA NITROGEN

It should be mentioned that the Ammonia determination listed on the right side of the flow chart follows the same procedure as the left side from DISTILL down to the completion.

The Organic Nitrogen may be calculated as follows:

Organic Nitrogen = Total Kjeldahl Nitrogen - Ammonia Nitrogen.

Equipment and Supply Requirements

A. Capital Equipment - Macro or Micro Determinations

1. Digestion Apparatus and Distillation Apparatus:
   The pieces of equipment required to assemble a system for digestion and distillation will differ according to whether a 500 ml sample (macro determination) or a 50 ml sample (micro determination) is analyzed. See the diagrams at the end of these listings to identify the items required for your choice of determinations.
2. Balance, analytical, capable of weighing to 0.1 mg at a 200 g load
3. Balance, triple beam, capable of weighing to 0.1 g at a 500 g load
4. Spectrophotometer for use at 400-425 nm with a light path of 1 cm or longer
5. Water Still and an anion-cation exchange system to produce ammonia-free water

B. Reusable Supplies - Macro or Micro Determinations

NOTE: All beakers and flasks should be either Pyrex® or Kimax®.

1. One 50 ml beaker, graduated
2. One 100 ml beaker, graduated
3. Two 150 ml beakers, graduated
4. One 250 ml beaker, graduated
5. One 50 ml bottle, glass with stopper
6. Three 150 ml bottles, glass with dropper tops
7. One 500 ml bottle, glass
8. Two 1000 ml bottles, glass with tops
9. One 50 ml buret
10. One 10 ml cylinder, graduated
11. One 100 ml cylinder, graduated
12. One 500 ml cylinder, graduated
13. One 50 ml Erlenmeyer flask, graduated
14. One 125 ml Erlenmeyer flask, graduated
15. Four 1000 ml Erlenmeyer flasks, graduated
16. Five 1000 ml volumetric flasks with stoppers
17. Glass beads, 4 mm
18. Nine Nessler tubes, scored at 50 ml
19. One Nessler tube support
20. Two 10 ml pipets, Mohr, graduated
21. One 10 ml pipet, volumetric
22. One 25 ml pipet, volumetric
23. One 50 ml pipet, volumetric
24. One ring stand
25. One buret holder
26. One #3 or #6 rubber stopper or a cap to fit Nessler tubes

C. Consumable Supplies - Macro or Micro Determinations

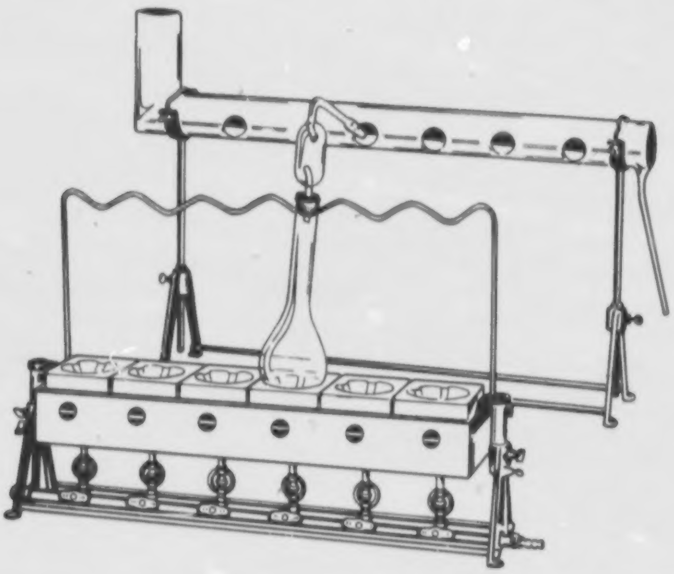
1. 4 g ammonium chloride, NH_4Cl, reagent, granular
2. 20 g boric acid, H_3BO_3,
3. 700 ml ethyl alcohol, C_2H_5OH, reagent,denatured
4. 0.5 g methyl orange, indicator (for titration method)
5. 200 mg methyl red, reagent (for titration method)
6. 200 mg methylene blue (for titration method)
7. 4 g mercuric oxide, HgO, reagent powder, red
8. 100 g mercuric iodide, HgI_2
9. 5 g phenolphthalein powder
10. 70 g potassium iodide, KI, reagent powder
11. 134 g potassium sulfate, K_2SO_4, reagent powder
12. 5 g sodium carbonate, Na_2CO_3, reagent powder (for titration method) anhydrous
13. 660 g sodium hydroxide, NaOH, reagent pellets
14. 25 g sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$, reagent grade
15. 223 ml sulfuric acid, H_2SO_4, reagent grade
16. 14 weighing boats

The following reagents may be purchased commercially thus alleviating several sections under reagent preparations and some of the above chemical requirements.

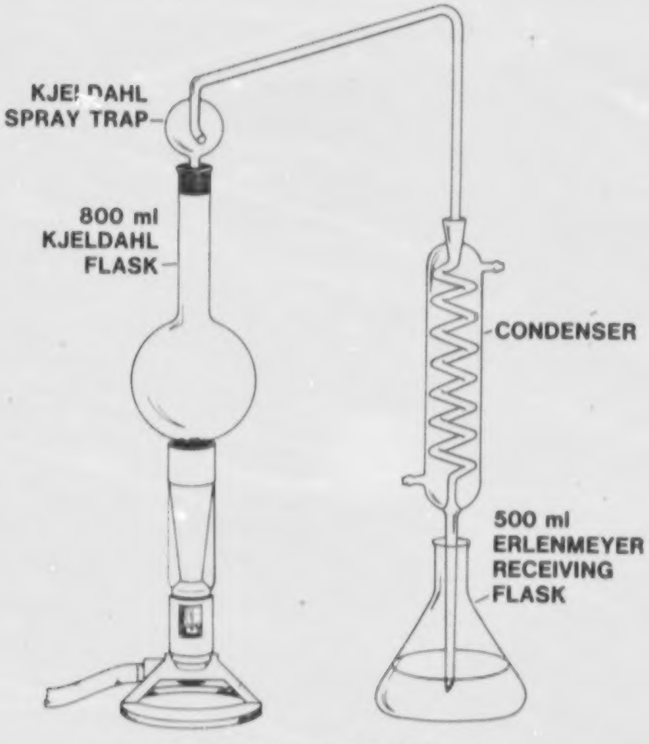
*1. Nessler reagent (100 g mercuric iodide, 70 g potassium iodide, 160 g sodium hydroxide)
*2. Phenolphthalein indicator solution, 1% (500 ml ethyl alcohol, 5 g phenolphthalein)
*3. Digestion reagent [i.e. Kel-Pac® (Olin-Matheson)] [4 g mercuric oxide (red), 134 g potassium sulfate, 220 ml sulfuric acid]
*4. Sulfuric acid (0.02N) (5 g sodium carbonate, 3 ml sulfuric acid)

*Commercially prepared reagents may be used in analyses for NPDES purposes if the solutions have been prepared according to the reagent section of the approved methods cited in the Federal Register. It is strongly recommended that purchased reagents be verified by initially checking them against a quality control check sample available through your Regional EPA Analytical Quality Control Coordinator (from 3/21/75 EPA-MDQARL memo).

MACRO KJELDAHL DETERMINATION

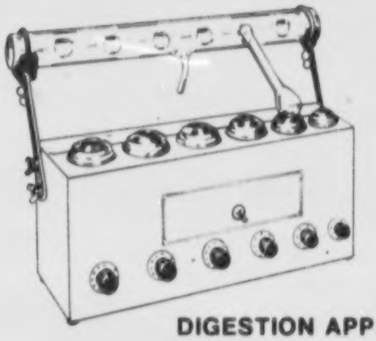


DIGESTION APPARATUS

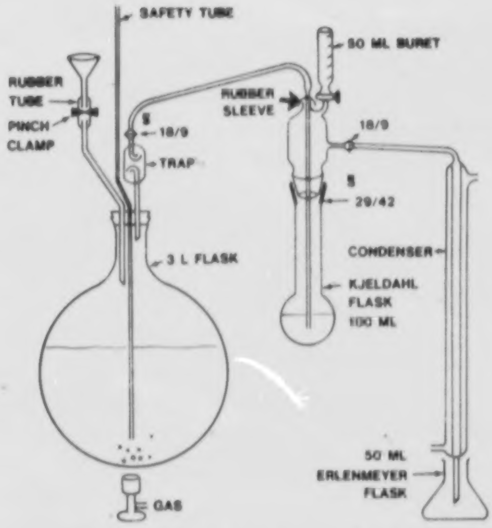

KJELDAHL SPRAY TRAP
800 ml KJELDAHL FLASK
CONDENSER
500 ml ERLENMEYER RECEIVING FLASK

DISTILLATION APPARATUS

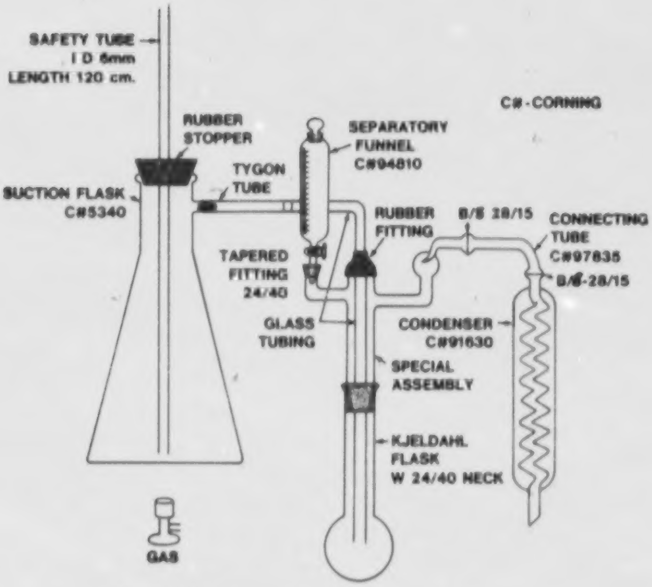
MICRO KJELDAHL DETERMINATION



DIGESTION APPARATUS


SAFETY TUBE
50 ML BURET
RUBBER TUBE
PINCH CLAMP
18/9
TRAP
RUBBER SLEEVE
18/9
29/42
3 L FLASK
CONDENSER
KJELDAHL FLASK 100 ML
50 ML ERLENMEYER FLASK
GAS

STEAM DISTILLATION APPARATUS


SAFETY TUBE I D 6mm LENGTH 120 cm.
C#-CORNING
RUBBER STOPPER
SEPARATORY FUNNEL C#94810
TYGON TUBE
SUCTION FLASK C#5340
RUBBER FITTING
B/S 28/15
CONNECTING TUBE C#97835
TAPERED FITTING 24/40
B/S-28/15
GLASS TUBING
CONDENSER C#91630
SPECIAL ASSEMBLY
KJELDAHL FLASK W 24/40 NECK
GAS

STEAM DISTILLATION APPARATUS

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
DETERMINATION OF TOTAL KJELDAHL NITROGEN			I (p. 36)
A. Equipment Preparation			
1. Glassware Washup	1. Clean all glassware in suitable detergent.	1a. Distilled water drains without leaving any droplets.	V.A.1.1a (p. 39)
2. Balance Inspection	1. Clean balance	1a. Free of dust and dirt.	
3. Spectrophotometer Inspection	1. Clean spectrophotometer.	1a. Free of dust and dirt.	V.A.3.1a (p. 39)
	2. Turn main power on by rotating the zero control clockwise.	2a. Pilot lamp on.	
	3. Select wavelength by rotating the knob at the extreme right on the top of the instrument either clockwise or counterclockwise.	3a. 425 nm.	
	4. Zero the instrument.	4a. Meter needle reads zero % T.	V.A.3.4a (p. 39)
	5. Use an empty cell and adjust the light control to 100% T.	5a. To be sure that the instrument can achieve 100% T.	V.A.3.5a (p. 39)
4. Still Cleaning	1. Add a 1:1 mixture of ammonia-free distilled water and sodium hydroxide-sodium thiosulfate solution to each of Kjeldahl flasks to be used.	1a. See Equipment and Supply Requirements Section for diagrams of Kjeldahl Apparatus. (Pages 5-8 & 5-9) 1b. Glass beads should be added to each flask. 1c. At least 400 ml of solution should be used for macro equipment. Use 40 ml for micro equipment. 1d. See B, Reagent Preparation #5.	V.A.4.1a (p. 39)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation (continued)	2. Using the appropriate apparatus, distill half of this solution.	2a. The distillate should be checked colorimetrically to insure that it is ammonia-free. The Nessler reagent that is used for the ammonia determination can be used at this point. (Reagent #15)	
	3. Add 1 ml of Nessler's Reagent to the distillate.	3a. If the distillate remains colorless, the glassware is not contaminated with ammonia. If the distillate turns yellow, distill another half and repeat step 3.	
B. Reagent Preparation			VI.B (p. 40)
1. Distilled Water	1. Prepare at least four (4) liters of distilled water. This water should be free from ammonia.	1a. All solutions must be made with ammonia-free water. It is best to have an ion exchange system in conjunction with a suitable water still to insure high quality water. An anion-cation exchange resin should be used.	VI.B.1.1a (p. 40)
2. Sulfuric Acid Solution (20% by volume)	1. Measure 50 ml of distilled water in a 125 ml Erlenmeyer flask.	1a. This solution is used in Reagent Preparation #3.	
	2. Add 20 ml of concentrated sulfuric acid (H_2SO_4) and mix.	2a. Flask should be tilted to avoid splattering. 2b. Solution may be diluted directly in the Erlenmeyer flask.	
	3. Dilute the solution to 100 ml.		
3. Mercuric Sulfate Solution	1. Weigh 4 grams of red mercuric oxide (HgO) in a weighing boat.	1a. This solution is used in Reagent Preparation #4.	
	2. Dissolve the mercuric oxide in 25 ml of the 20% sulfuric acid solution.	2a. A 100 ml beaker may be used.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	3. Dilute the solution to 50 ml with distilled water.		
4. Digestion Reagent	1. Weigh 134 grams of potassium sulfate (K_2SO_4).	1a. A 150 ml beaker is suitable for this weighing.	
	2. Add 650 ml of distilled water to a 1 liter Erlenmeyer flask.		
	3. Add 200 ml of concentrated sulfuric acid (H_2SO_4) and mix.	3a. Erlenmeyer flask should be tilted to avoid splattering. 3b. Caution: Solution and flask tend to become warm. A cold water bath may be used to keep the temperature down.	
	4. Dissolve the potassium sulfate in this solution.		
	5. Add 25 ml of the mercuric sulfate solution (reagent 3) to the solution and mix.		
	6. Dilute the solution to 1 liter.	6a. Store in glass container. 6b. The solution should be kept at about 14°C to prevent crystallization. 6c. If crystals form, warm the solution in the flask on a hot plate and stir/swirl to re-dissolve the crystals.	
5. Sodium Hydroxide-Sodium Thiosulfate Solution	1. Weigh 500 grams of sodium hydroxide (NaOH) and 25 grams of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) in a 1 liter Erlenmeyer flask.	1a. Exercise caution with such a large amount of sodium hydroxide since this is a very caustic substance. 1b. Double check for pyrex glassware.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	2. Add approximately 700 ml of distilled water and dissolve the reagents.	2a. Fumes will be given off. Therefore use a suitable venting device.	
	3. Dilute to 1 liter with distilled water.	3a. Cool to room temperature before diluting to final volume.	
6. Phenolphthalein Indicator Solution (0.5%)	1. Weigh 5 grams of phenolphthalein in a weighing boat.		
	2. Dissolve in 500 ml of 95% ethyl alcohol in a glass bottle or container.		
	3. Add 0.02 N NaOH until a faint pink color appears.	3a. Dissolve 0.4 g NaOH in 500 ml of ammonia-free distilled water to make 0.02 N NaOH. Very exact weighing is not necessary.	
	4. Store in a glass or plastic bottle.		
7. Methyl Red Indicator Solution (0.2%)	1. Weigh 200 mg of methyl red indicator in a 150 ml beaker.		
	2. Add 100 ml of 95% ethyl alcohol and dissolve the indicator.	2a. This solution will be used to prepare #9, mixed indicator which is required for the titrimetric method to determine ammonia.	
8. Methylene Blue Indicator Solution (0.2%)	1. Weigh 200 mg of methylene blue indicator in a 150 ml beaker.		
	2. Add 100 ml of 95% ethyl alcohol and dissolve the indicator.	2a. This solution will be used to prepare #9, mixed indicator.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
9. Mixed Indicator	1. Mix 100 ml of the methyl indicator solution with 50 ml of the methylene blue indicator solution.	1a. The mixed indicator is required for the titrimetric method to determine ammonia. 1b. This solution should be prepared fresh every 30 days.	
10. Methyl Orange Indicator Solution	1. Weigh 100 mg of methyl orange indicator in a 150 ml beaker.	1a. This indicator is required for the titrimetric method to determine ammonia.	
	2. Add 100 ml ammonia-free distilled water.		
	3. Stir to dissolve the indicator.	3a. If the solution is cloudy, filter it.	
	4. Store in a 150 ml glass bottle with dropper top.		
11. Boric Acid Solution	1. Weigh 20 grams of boric acid (H_3BO_3) in a weighing boat.		
	2. Transfer to a 1 liter Erlenmeyer flask and dilute the acid to 1 liter.		
12. Ammonium Chloride Stock Solution	1. Weigh 3.819 grams of ammonium chloride (NH_4Cl) in a weighing boat.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	2. Dissolve the ammonium chloride in ammonia-free distilled water in a 1 liter volumetric flask.		
	3. Dilute to 1 liter.	3a. 1 ml - 1.0 mg Ammonia Nitrogen (NH_3-N).	
13. Ammonium Chloride Standard Solution	1. Dilute 10.0 ml of the stock solution to 1 liter in a volumetric flask.	1a. Use a volumetric pipet. 1b. 1 ml - 0.01 mg ammonia nitrogen (NH_3-N).	
14. Sodium Hydroxide Solution	1. Weigh 160 grams of sodium hydroxide (NaOH) in a 1 liter Erlenmeyer flask.	1a. This solution is used in Reagent Preparation #15 which is required for the colorimetric (Nessler) method to determine ammonia.	
	2. Add 500 ml of ammonia-free, distilled water.		
	3. Dissolve the sodium hydroxide, and cool to room temperature.		
15. Nessler Reagent	1. Weigh 100 grams of mercuric iodide (HgI_2) and 70 grams of potassium iodide (KI) together in a 250 ml beaker.		

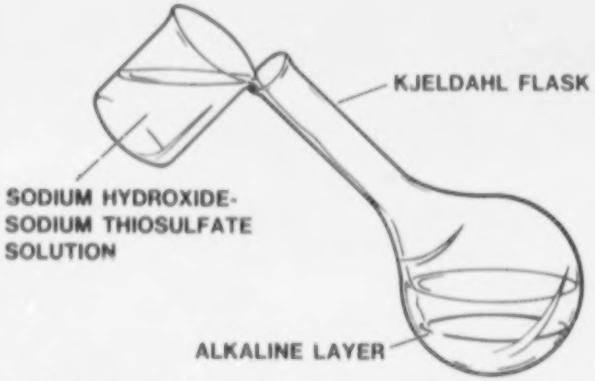
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	2. Add enough distilled water to dissolve the mixture.	2a. Approximately 50 ml should be sufficient.	
	3. Add this mixture slowly with stirring to the sodium hydroxide solution (#14).		
	4. Dilute the mixture to 1 liter.	4a. The solution is stable for at least one year in a pyrex bottle out of direct sunlight.	
C. Preparation and Standardization of 0.02 N Sulfuric Acid Titrant		Ca. This entire procedure is required only if you are using the titrimetric method to determine ammonia.	
1. Sulfuric Acid, Approximately 0.1 N	1. Add 3 ml of concentrated sulfuric acid (H_2SO_4) to 600 ml of carbon dioxide-free water in a 1 liter volumetric flask.	1a. Heat 2 liters of distilled water for 15 minutes to drive off the carbon dioxide (CO_2).	
	2. Dilute this solution to 1 liter.		
2. Sulfuric Acid, Approximately 0.02 N	1. Dilute 200 ml of the 0.1 N sulfuric acid solution to 1 liter in a volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Preparation and Standardization of 0.02 N Sulfuric Acid Titrant (continued)			
3. Sodium Carbonate Standard, 0.0200 N	1. Dry 5 grams of sodium carbonate (Na_2CO_3) at 140°C for 2 hours.		
	2. Cool in a desiccator.	2a. Thirty minutes is recommended.	
	3. Weigh 1.060 grams in a weighing boat.	3a. Use the analytical balance.	
	4. Transfer to 1 liter volumetric flask.		
	5. Dissolve the salt and dilute to 1 liter with carbon dioxide-free water.	5a. See C.1.1a for preparation of carbon dioxide - free water.	
4. Standardization of the Sulfuric Acid Solution	1. Fill a 50 ml buret with approximately 0.02 N sulfuric acid solution.		
	2. Transfer 25.0 ml of the sodium carbonate solution to a 125 ml Erlenmeyer flask.	2a. Transfer with 25.0 ml volumetric pipet. 2b. A beaker may be used, if a magnetic stirrer is available.	
	3. Add 2 drops of a methyl orange indicator to the flask.		

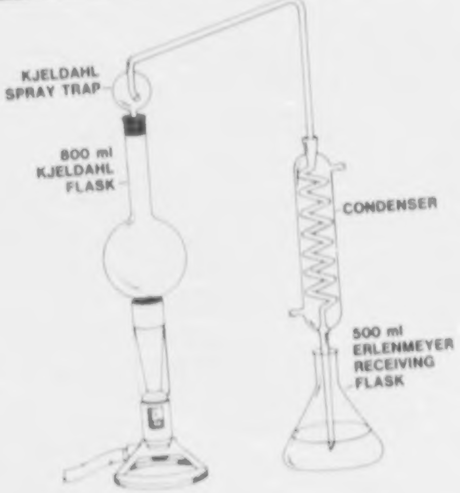
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Preparation and Standardization of 0.02 N Sulfuric Acid Titrant (continued)	4. Add the approximately 0.02 N sulfuric acid solution to the sodium carbonate solution until the color changes from yellow to orange.	4a. A pink color indicates the titration has gone too far and should be repeated.	
	5. Record the ml of sulfuric acid used.		
	6. Calculate the normality of the sulfuric acid titrant.	6a. Example Calculation If the number of ml used is 20.8, the calculations would be as follows: $N_{H_2SO_4} = \frac{N_{Na_2CO_3} \times V_{Na_2CO_3}}{V_{H_2SO_4}}$ $N_{H_2SO_4} = \frac{0.0200\ N \times 25.0}{20.8}$ $N_{H_2SO_4} = \frac{0.500}{20.8}$ $N_{H_2SO_4} = \underline{\underline{0.0240\ N}}$	
264	7. Record the correct normality on the storage bottle.	7a. For example, the above 0.0240 N value should be recorded on the storage bottle for the sulfuric acid titrant.	265

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>D. Analysis Using Macro Apparatus (800 ml Flasks)
See Procedure E for Analysis Using Micro Apparatus (100 ml Flasks), page 23</td><td></td><td>D.a. See diagrams of Macro Apparatus in the Section on Equipment and Supply Requirements. (Page 5-8).</td><td></td></tr>
<tr><td>1. Measurement of Sample</td><td>1. Place a measured amount of well-shaken sample into an 800 ml Kjeldahl flask.</td><td>1a. Sample size can be determined from the following table:
<table>
<tr><th>Kjeldahl Nitrogen in Sample; mg/liter</th><th>Sample Size ml</th></tr>
<tr><td>0-5</td><td>500</td></tr>
<tr><td>5-10</td><td>250</td></tr>
<tr><td>10-20</td><td>100</td></tr>
<tr><td>20-50</td><td>50.0</td></tr>
<tr><td>50-500</td><td>25.0</td></tr>
</table>
1b. A normal effluent should have an organic nitrogen concentration between (0) and (1) mg/liter. If it is known that the concentration is greater than 1 mg/liter, the sample volume should be adjusted appropriately.
1c. Record information about the sample and the "ml sample used" on an appropriate data sheet. See Training Guide.</td><td>IX.D.1.1c. (p. 41)</td></tr>
<tr><td></td><td>2. If the sample size is less than 500 ml, dilute to 500 ml with distilled water.</td><td>2a. Use a graduated cylinder to measure the difference in volume.</td><td></td></tr>
<tr><td></td><td>3. Add several glass beads.</td><td>3a. Glass beads should prevent bumping in the flask.</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Analysis Using Macro Apparatus (800 ml Flasks) (continued)			
2. Reagent Addition	1. Add 100 ml of the digestion reagent to the flask.	1a. Use a graduated cylinder for the digestion reagent prepared in B.4. 1b. If commercially available packets are used, then 1 packet (for macro Kjeldahl digestions) would be added in place of the reagent.	VI.D.2.1a (p. 40)
3. Digestion	1. Evaporate the mixture in the Kjeldahl apparatus until sulfur trioxide (SO_3) fumes are given off and the solution turns pale yellow.	1a. See diagram in Section on Equipment and Supply Requirements for proper position in digestion rack. (Page 5-8) 1b. SO_3 fumes will be indicated when white smoke begins rising from the solution. 1c. Sulfur trioxide (SO_3) fumes are extremely toxic. Therefore extreme caution should be observed.	
	2. Continue heating for 30 additional minutes.		
	3. Cool the residue.		
4. Distillation	1. Add 300 ml of ammonia-free, distilled water to the digested mixture in the Kjeldahl flask.		
	2. Add 0.5 ml of the phenolphthalein indicator solution.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Analysis Using Macro Apparatus (800 ml Flasks) (continued)	3. Add 50 ml of the 2% boric acid to a 500 ml Erlenmeyer receiving flask.	3a. Before using the flask, measure 350 ml of ammonia-free distilled water in a graduate, pour it into the flask and make a mark at 350 ml on the outside. You will need this marking for a later step.	
	4. Position the Erlenmeyer flask so that the tip of the condenser (or an extension of the condenser tip) is below the level of the boric acid solution in the receiving flask. (See diagram next to Step 6 below)		
	5. Tilt the flask and carefully add 100 ml of the sodium hydroxide-thiosulfate solution to form an alkaline layer at the bottom of the flask. (See diagram at right).	 KJELDAHL FLASK SODIUM HYDROXIDE-SODIUM THIOSULFATE SOLUTION ALKALINE LAYER 5a. The lower layer should be red. 5b. Do not agitate the digestion flask until it is connected to the distillation apparatus, since free ammonia may be liberated too soon.	

EFFLUENT MONITORING PROCEDURE: Determination of Total Kjeldahl Nitrogen

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Analysis Using Macro Apparatus (800 ml Flasks) (continued)	6. Connect the Kjeldahl flask to the condenser. (See diagram at right)	 KJELDAHL SPRAY TRAP 800 ml KJELDAHL FLASK CONDENSER 500 ml ERLENMEYER RECEIVING FLASK	
	7. Turn on the heat source.		
	8. Distill up to the 350 ml mark on the Erlenmeyer flask at the rate of 6-10 ml/min into the boric acid solution.		
	9. Remove the receiving flask.		
	10. Put a small beaker under the condenser tip.	10a. To receive any additional distillate.	
	11. Remove the heat source.	11a. To stop the distillation.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Analysis Using Macro Apparatus (800 ml Flasks) (continued)	12. Dilute the distillate to 500 ml by adding ammonia-free distilled water up to the 500 ml mark on the flask.	12a. Record 500 ml on the data sheet as "B. ml total distillate, including boric acid (H_3BO_3) and dilution water."	IX.D.4.12a (p. 41)
5. Determining Ammonia	1. If the sample is a normal effluent, the anticipated nitrogen concentration of 0-1 mg/liter requires the Colorimetric Method presented as Procedure F. 2. If it is known that the nitrogen concentration is greater than 1 mg/liter, use the Titrimetric Method presented as Procedure G.		
E. Analysis Using Micro Apparatus (100 ml Flasks)		Ea. See diagram of Micro Apparatus in the Section on Equipment and Supply Requirements. (Page 5-9)	
1. Measurement of Sample	1. Place a measured amount of well-shaken sample into a 100 ml Kjeldahl flask.	1a. Sample size can be determined from the following table:	

Kjeldahl Nitrogen in Sample; mg/l	Sample Size ml
0-5	50
5-10	25
10-20	10
20-50	5
50-500	2

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Analysis Using Micro Apparatus (100 ml Flasks) (continued)		1b. A normal effluent should have an organic nitrogen concentration between (0) and (1) mg/l. If it is known that the concentration is greater than 1 mg/l, the sample volume should be adjusted appropriately. 1c. Record information about the sample and the "ml sample used" on an appropriate data sheet. See Training Guide.	IX.E.1.1c (p. 41)
	2. If sample size is less than 50 ml, dilute to 50 ml with distilled water.	2a. Use a graduated cylinder to measure the difference in volume.	
	3. Add several glass beads.	3a. Glass beads should prevent bumping in the flask.	
2. Reagent Addition	1. Add 10 ml of the digestion reagent to the flask.	1a. Use a graduated cylinder for the digestion reagent prepared in B.4. 1b. If commercially available packets are used, then 1 packet (for micro Kjeldahl digestion) would be added in place of the reagent.	VI.E.2.1a (p. 40)
3. Digestion	1. Evaporate the mixture in Kjeldahl apparatus until sulfur trioxide (SO_3) fumes are given off and the solution turns pale yellow.	1a. See diagram in Section on Equipment and Supply Requirements for proper position in digestion rack. (page 5-9) 1b. SO_3 fumes will be indicated when white smoke begins rising from the solution. 1c. Sulfur trioxide (SO_3) fumes are extremely toxic. Therefore extreme caution should be observed.	
	2. Continue heating for an additional 30 minutes.		
	3. Cool the residue.		

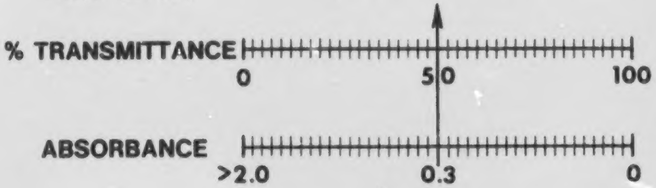
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Analysis Using Micro Apparatus (100 ml Flasks) (continued)	1. Add 30 ml of ammonia-free distilled water to the digested mixture in the Kjeldahl flask.		
4. Steam Distillation	2. Add 2 drops of the phenolphthalein indicator solution.		
	3. Connect the Kjeldahl flask to the ground glass joint of the Micro steam distillation apparatus.	3a. Diagrams of this apparatus are in the section on Equipment and Supply Requirements. (page 5-9)	
	4. Add 5 ml of the 2% boric acid to a 50 ml Erlenmeyer receiving flask.	4a. A 50 ml short-form Nessler tube also may be used. 4b. Before using the flask or Nessler tube, measure 35 ml of ammonia-free distilled water in a graduate, pour it into the receiving container and make a mark at 35 ml on the outside. You will need this marking for a later step.	
	5. Position the receiving flask so that the tip of the condenser (or an extension of the condenser tip) is below the level of the boric acid solution in the receiving flask.		
	6. Carefully add 10 ml of the sodium hydroxide-thiosulfate solution from the dropping funnel.	6a. The mixture in the Kjeldahl flask should be red.	
	7. Turn on the heat source.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Analysis Using Micro Apparatus (100 ml Flasks) (continued)	8. Distill up to the 35 ml mark on the receiving flask at the rate of 6-10 ml/min, into the boric acid solution.	8a. Exercise caution when working with this steam apparatus.	
	9. Remove the receiving flask.	9a. If an Erlenmeyer receiver was used, transfer the distillate to a 50 ml Nessler Tube now.	
	10. Put a small beaker under the condenser tip.	10a. To receive any additional distillate.	
	11. Remove the heat source.	11a. To stop the distillation.	
	12. Dilute the distillate to 50 ml by adding ammonia-free distilled water up to the 50 ml mark on the Nessler tube.	12a. Record 50 ml on the data sheet as "B. ml total distillate, including boric acid (H_3BO_3) and dilution water."	IX.E.4.12a (p. 41)
5. Determining Ammonia	1. If the sample is a normal effluent, the anticipated nitrogen concentration of 0-1 mg/liter requires the Colorimetric Method presented as Procedure F.		
	2. If it is known that the nitrogen concentration is greater than 1 mg/liter, use the Titrimetric Method presented as Procedure G.		
F. Colorimetric Method			
1. Color Development of Standards and Sample	1. Place nine Nessler tubes in the Nessler support rack.		
	2. Label the tubes (1-9).	2a. Use small stick-on labels.	

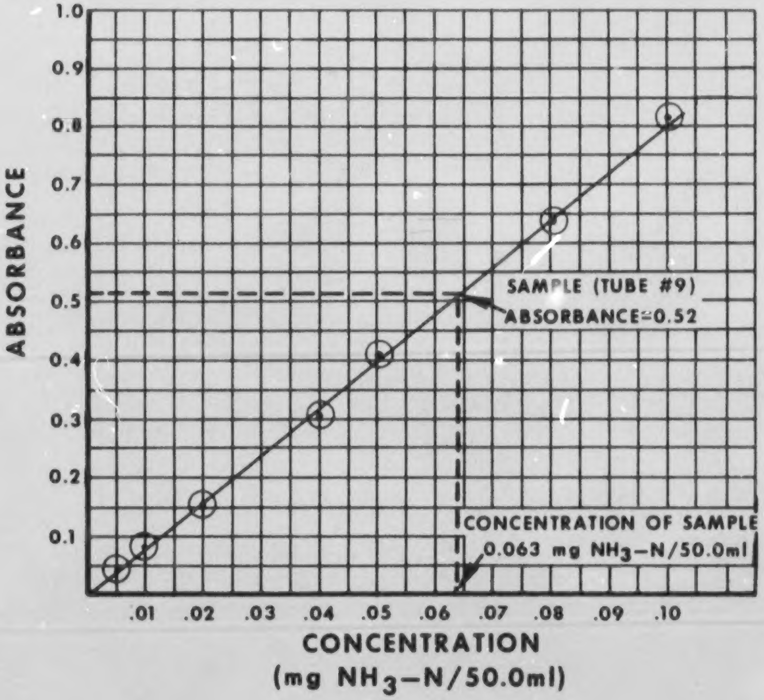
<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>F. Colorimetric Method (continued)</td><td>3. The Nessler tubes will be used for preparing the standards for the determination. The table on the right lists the volumes of standard ammonia solution to be added to each tube. The volumes of standard should be measured with a Mohr pipet.</td><td>3a.
<table>
<tr><th>Tube #</th><th>ml of standard Ammonia Solution</th><th>mg of Ammonia Nitrogen per 50.0 ml</th></tr>
<tr><td>1</td><td>0.0</td><td>0.0</td></tr>
<tr><td>2</td><td>0.5</td><td>0.005</td></tr>
<tr><td>3</td><td>1.0</td><td>0.010</td></tr>
<tr><td>4</td><td>2.0</td><td>0.020</td></tr>
<tr><td>5</td><td>4.0</td><td>0.040</td></tr>
<tr><td>6</td><td>5.0</td><td>0.050</td></tr>
<tr><td>7</td><td>8.0</td><td>0.080</td></tr>
<tr><td>8</td><td>10.0</td><td>0.10</td></tr>
</table></td><td></td></tr>
<tr><td></td><td>4. Add ammonia-free distilled water to each tube, diluting each to the 50 ml line.</td><td></td><td></td></tr>
<tr><td></td><td>5. Into tube #9 place 50 ml of the sample taken from the receiving flask containing distillate.</td><td>5a. If Macro apparatus was used, pour the distillate from D.4.12 into tube #9 up to the 50.0 ml mark.
5b. If Micro apparatus was used, the distillate was either collected in a 50 ml Nessler tube or else transferred to one after distillation. The distillate was diluted to 50 ml in step E.4.12. Label this Nessler tube as #9 for these steps.
5c. In either case, record 50 ml on the data sheet as "C. ml distillate taken for Nesslerization."</td><td>IX.F.1.5c (p. 41)</td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Colorimetric Method (continued)	6. Add 1 ml of Nessler Reagent to each standard and sample.	6a. Use a Mohr Pipet.	
	7. Mix the solution by placing a cap on the tube and inverting three times.	7a. A #3 or a #6 rubber stopper may be used instead of a cap. 7b. Rinse and dry the cap or stopper after each use with a tube.	
	8. Place the tubes back into the rack and let sit for 20 minutes.	8a. During this time span, the spectrophotometer should be double checked for proper operation. (see Spectrophotometric Inspection in A.3.)	
2. Spectrophotometric Measurements	1. Arrange 9 spectrophotometric tubes (1/2") in a test tube rack and label 1-9.	1a. If you do not have a matched set of tubes for your spectrophotometer, a single tube can be used. It should be rinsed with distilled water, then with the solution to be put into the instrument. The procedure is presented in the EMP, "Use of a Spectrophotometer".	
	2. After the twenty minute time span, transfer the appropriate standards and sample to these tubes.		
	3. Place tube (#1) in the sample holder of the instrument.	3a. The wavelength should be set at 425 nm.	
	4. Using the light control turn the knob until the meter needle reads 100% on the transmittance (T) scale.		

EFFLUENT MONITORING PROCEDURE: Determination of Total Kjeldahl Nitrogen

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Colorimetric Method (continued)	5. Place tube #2 in the sample holder and record the absorbance value.	5a. This value will be found on the bottom scale as shown below.  % TRANSMITTANCE 0 50 100 ABSORBANCE >2.0 0.3 0 For example the transmittance was 50%, the absorbance should be recorded as 0.3 A.	
	6. Place tubes #3 through #9 in the sample holder, recording each absorbance value.	6a. Values should be recorded in a notebook or on a appropriate data sheet. See Training Guide. 6b. EXAMPLE RECORD OF ABSORBANCE VALUES	IX.F.2.6a (p. 42)

Tube #	Concentration (mg NH_3-N/50.0 ml)	Absorbance
1	0.0	0.0
2	0.005	0.04
3	0.010	0.08
4	0.020	0.16
5	0.040	0.32
6	0.050	0.41
7	0.080	0.64
8	0.10	0.82
9	To be determined from calibration curve.	0.52

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Colorimetric Method (continued)			
3. Plotting and use of the calibration curve.	1. Plot the absorbance values for the standards obtained in F2. above vs. the concentration of ammonia nitrogen in the standards as in the Table in F.1.3.3a.	1a. Following, below, there is an example calibration curve using the example absorbances from the table in F.2.6b above vs the concentration of ammonia nitrogen in the standards as in the Table in F.1.3.3a. The absorbance value used for the sample is 0.52.  	
	2. Draw the best straight line through all the points to produce a calibration curve.		
	3. Use the absorbance value for the sample (Tube #9) obtained in F.2.6.6b. above to draw a dotted line from the absorbance line over to the calibration curve.		
	4. From that point on the calibration curve, draw a perpendicular line down to the concentration line.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Colorimetric Method (continued)	5. Record the concentration value at this point for sample as mg NH_3-N/50.0 ml.	5a. Record this on the data sheet as "A, mg NH_3-N/50.0 ml from curve." 5b. In this example, the concentration for the sample is 0.063 mg NH_3-N/50.0 ml.	IX.F.3.5a (p. 41)
4. Final Calculation for Macro Analysis (See next procedure, #5, for Final Calculation for Micro Analysis)	1. Using the formula at the right, compute the Total Kjeldahl Nitrogen concentration.	1a. TKN, mg/l = $\frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$ Where: A = mg NH_3-N (ammonia nitrogen)/50.0 ml from curve B = ml total distillate, including boric acid (H_3BO_3) and dilution water C = ml distillate taken for Nesslerization ml sample = ml of original sample taken An example calculation using a value from a calibration curve would be: TKN, mg/l = $\frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$ A = 0.044 B = 500 ml (300 ml distillate + 50 ml boric acid + 150 ml dilution water) C = 50 ml ml sample = 500 ml TKN, mg/l = $\frac{0.044 \times \overset{2}{\cancel{1000}}}{\underset{1}{\cancel{500}}} \times \frac{\overset{10}{\cancel{500}}}{\underset{1}{\cancel{50}}}$ = 0.044 X 2 X 10 = 0.044 X 20 = 0.88 TKN = 0.88 mg/l	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Colorimetric Method (continued)			
5. Final Calculation for Micro Analysis	1. Using the formula at the right, compute the Total Kjeldahl Nitrogen concentration.	1a. TKN, mg/l = $\frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$ Where: A = mg NH_3-N (ammonia nitrogen)/50.0 ml from curve B = ml total distillate, including boric acid (H_3BO_3) and dilution water C = ml distillate taken for Nesslerization ml sample = ml of original sample taken An example calculation using a value from a calibration curve would be: TKN, mg/l = $\frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$ A = 0.045 B = 50 ml (30 ml distillate + 5 ml boric acid + + 15 ml dilution water) C = 50 ml ml sample = 50 ml TKN, mg/l = $\frac{0.045 \times \overset{20}{\cancel{1000}}}{\underset{1}{\cancel{50}}} \times \frac{\overset{1}{\cancel{50}}}{\underset{1}{\cancel{50}}}$ = 0.045 X 20 X 1 = 0.045 X 20 = 0.90 TKN = 0.90 mg/l	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Titrimetric Method			
1. Titration	1. Transfer the contents of the distillate receiving flask (from D.4.12 or E.4.12) to the next largest volume Erlenmeyer titration flask.		
	2. Add 3 drops of mixed indicator to the flask and its contents.	2a. If ammonia nitrogen is present the color of the solution will be green.	
	3. Set up a buret for titration.	3a. Use a 50 ml buret.	
	4. Fill the buret with a 0.020 N sulfuric acid (H_2SO_4) standard solution.		
	5. Add the sulfuric acid titrant until the color of the solution changes from green to purple.	5a. A blank can be analyzed also, so that the true color can be seen. 5b. A blank contains all necessary reagents except distilled water is substituted for the sample.	
2. Calculations	1. The Total Kjeldahl Nitrogen (TKN) would be calculated by the formula to the right.	1a. TKN mg/l = $\frac{(A-B)N \times F \times 1000}{S}$ A = ml of standard H_2SO_4 used in titrating sample B = ml of standard H_2SO_4 used in titrating blank N = normality of sulfuric acid (Procedure C.) F = 14 (the millequivalent weight of nitrogen) S = ml of sample digested If the Normality (N) of the Sulfuric Acid is exactly 0.020 N then the formula may be reduced to TKN mg/l = $\frac{(A-B) \times 280}{S}$ where A, B, S, refer to the same terms as above.	

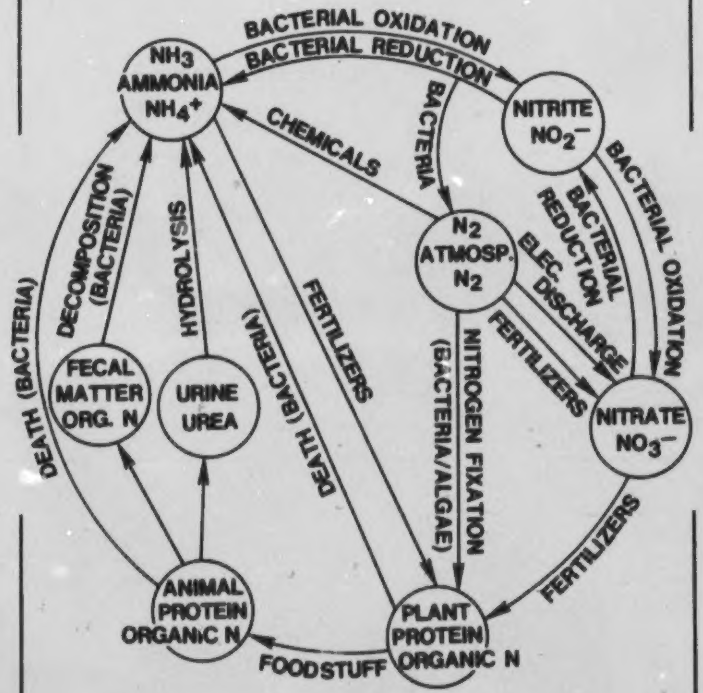
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Titrimetric Method (continued)	2. Given the following sample data, the computation would be made as shown at the right. A = 19.2 ml B = 0.4 ml N = 0.021 F = 14 S = 500 ml	2a. EXAMPLE CALCULATION: $\text{TKN, mg/l} = \frac{(A-B)N \text{ X } F \text{ X } 1000}{S}$ $= \frac{(19.2 - 0.4) \text{ X } 0.021 \text{ X } 14 \text{ X } 1000}{500}$ $= \frac{18.8 \text{ X } 0.021 \text{ X } 14 \text{ X } \overset{2}{\cancel{1000}}}{\underset{1}{\cancel{500}}}$ $= 18.8 \text{ X } 0.021 \text{ X } 14 \text{ X } 2$ $= 18.8 \text{ X } 0.021 \text{ X } 28$ $= 18.8 \text{ X } 0.588$ $= 11.1 \text{ mg/l}$ $\text{TKN, mg/l} = \underline{\underline{11.1 \text{ mg/l}}}$	

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts-Mathematics
III	Educational Concepts-Science
IV	Educational Concepts-Communications
V*	Field and Laboratory Equipment
VI*	Field and Laboratory Reagents
VII	Field and Laboratory Analysis
VIII	Safety
IX*	Records & Reports

Training guide materials are presented here under the headings marked. These standardized headings are used throughout this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	Nitrogen has long been recognized as a very important element in regard to pollution control analysis. Nitrogen can exist in several forms. These forms are related by what is called the nitrogen cycle (shown below).	Chemistry for Sanitary Engineers, Sawyer and McCarty, 2nd Edition, McGraw-Hill, 1967.


BACTERIAL OXIDATION
BACTERIAL REDUCTION
NH3 AMMONIA NH4+
NITRITE NO2−
BACTERIA
CHEMICALS
BACTERIAL OXIDATION
BACTERIAL REDUCTION
N2 ATMOSP. N2
ELEC. DISCHARGE
FERTILIZERS
NITRATE NO3−
DEATH (BACTERIA)
DECOMPOSITION (BACTERIA)
HYDROLYSIS
FECAL MATTER ORG. N
URINE UREA
DEATH (BACTERIA)
FERTILIZERS
NITROGEN FIXATION (BACTERIA/ALGAE)
FERTILIZERS
ANIMAL PROTEIN ORGANIC N
PLANT PROTEIN ORGANIC N
FOODSTUFF

THE NITROGEN CYCLE

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

Looking at the diagram one can see that the atmosphere contains a large amount of nitrogen existing as (N_2). The atmospheric nitrogen is converted to N_2O_5 in an electrical storm. This nitrogen pentoxide is subsequently converted to nitrates, $(NO_3)^-$, as a result of the mixing with water. These nitrates serve as fertilizer for plants and are subsequently converted to plant protein.

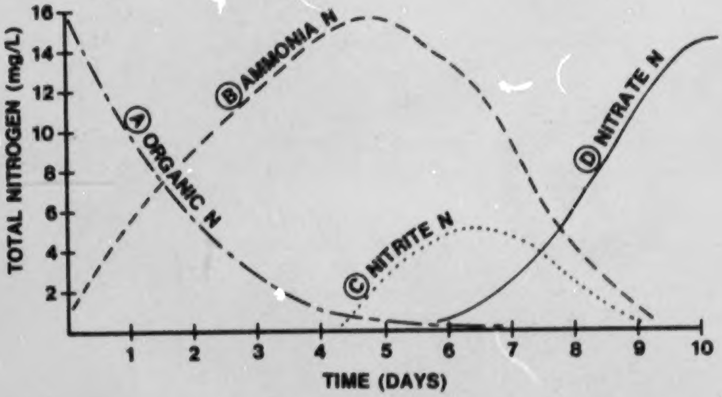
Animals and human beings utilize plant protein for the growth and repair of muscle tissue as well as energy. These nitrogen compounds are subsequently discharged as waste products (fecal matter and urine). Bacterial decomposition of fecal matter as well as hydrolysis of urine will produce ammonia. The bacterial decomposition may be accomplished under aerobic or anaerobic conditions.

The ammonia formed by this process may now further undergo bacterial oxidation (aerobic conditions) to form nitrites, $(NO_2)^-$, and eventually nitrates, $(NO_3)^-$, which can be used as fertilizer for plants etc.

It should be noted that several changes may occur that will modify the fate of a certain compound in the cycle. For example the system suddenly turns anaerobic upon nitrate, $(NO_3)^-$, formation. This would cause bacterial reduction to occur. Several other examples are shown in the cycle.

The treatment plant utilizes the nitrogen cycle in its processes. The raw sewage will have somewhat high Organic Nitrogen content. As it moves through the treatment process, it is converted to ammonia, nitrites and finally nitrates. An example of the Nitrogen Transformation in a typical treatment system is shown on the next page.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES



Isolating a certain volume of raw sewage on Day I (Point A), one can see that the Organic Nitrogen is relatively high but decreases and is converted to ammonia (Point B). Subsequent bacterial oxidation produces nitrites (Point C) and finally nitrates (Point D).

It therefore can be seen that the treatment plant simply follows the nitrogen cycle, and with proper monitoring procedures, (analysis of these 4 parameters), one can very easily measure the efficiency of the treatment process.

TRAINING GUIDE NOTE	REFERENCES/RESOURCES
The test described in this instruction can be found in the 1974 EPA Methods Manual on page 175. Another reference with an acceptable procedure for NPDES purposes is 14th ed. Standard Methods on page 437.	Methods for Chemical Analysis of Water and Wastes, 1974, EPA, MDQARL, Cincinnati, OH 45268, p. 175. Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, NY, p. 437.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1a	Most glassware can be cleaned simply by washing them first with detergent such as Alconox. Next rinse with tap water and finally with distilled water. For glass stopcocks, wrap the glass plug in tissue during storage. If a film or droplets appear on the glassware, use an Acid-Potassium Dichromate cleaning solution.	U.S. EPA Handbook for Analytical Quality Control in Water and Wastewater Laboratories, 1972, AQCL-NERC, Cincinnati, Ohio "Guidelines to the Care and Use of Analytical Glassware and Apparatus" Hudson Champlain Project
A.3.1a	The EMP written for the operation of this instrument should be consulted for further detail. The "Spectronic 20" operates on the principle that visible light broken down into all wavelengths may be used in quantitative determinations.	
A.3.4a	After 10 minutes warm-up time, the zero control may be adjusted to bring the meter needle to "0" on the percent transmittance (%T) scale.	
A.3.5a	Later on during the procedure a reagent blank is used for the final adjustment of the light control.	
A.4.1a	The O&M manual for the appropriate digestion rack should be consulted before operation. A macro Kjeldahl distillation apparatus utilizes an 800 ml flask which requires 500 ml of sample. A micro Kjeldahl apparatus utilizes a 100 ml flask which requires 50 ml of sample. Either Apparatus is acceptable.	

LABORATORY REAGENTS Section VI

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B	If Organic Nitrogen concentrations are known to be below 1 mg/l then the following reagents may be eliminated from procedure. 1. Methyl Red Indicator Solution - B.7 2. Methylene Blue Indicator Solution - B.8 3. Mixed Indicator - B.9 4. Methyl Orange Indicator Solution - B.10 5. Sulfuric Acid Titrant - All of Section C	
B.1.1a	High Ammonia (NH_3) concentration in distilled water could possibly influence the expected low values for normal nitrogen content of effluents.	
D.2.1a E.2.1a	Disposal of mercury-containing samples is a recognized problem; research investigations are under way to replace it as a preservative.	Dean, Williams, Wise: "Disposal of Mercury Wastes from Water Laboratories," Environmental Science and Technology, Vol. 5, No. 10, 1971. p. 1044 Maag and Hecker: "Recovery of Mercury in Solution," Journal of Environmental Quality, Vol. 1, No. 2, 1972, p. 192.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	Typical Laboratory Data Sheet for Total Kjeldahl Nitrogen, mg/l Name of Plant ______________	
D.1.1c. E.1.1c.	Sampling Location ______________ Type of Sample ______________ Date and Time Collected ______________ Sample Collector ______________ Date and Time Analysis Began ______________ Analyst ______________ Method Used (Macro or Micro) ______________ ml. sample used....................................	______________
D.4.12a E.4.12a	B. ml total distillate including boric acid (H_3BO_3) and dilution water..............................	______________
F.1.5c.	C. ml distillate taken for Nesslerization...........	______________
F.3.5a.	A. mg NH_3-N/50.0 ml, from curve.....................	______________

Use this formula in calculating the results for the colorimetric method:

$$\text{TKN mg/l} = \frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$$ (See pp. 5-31 and 5-32)

If Organic Nitrogen (mg/l) is needed and a separate ammonia analysis has been performed, use the following equation to determine this.

Since: TKN = Organic/N + Ammonia/N,

Then: Organic/N = TKN - Ammonia/N

Final Results

TKN mg/l ______________

NH_3-N, mg/l ______________

Org-N, mg/l ______________

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.2.6a	Values from Nesslerization Procedure	

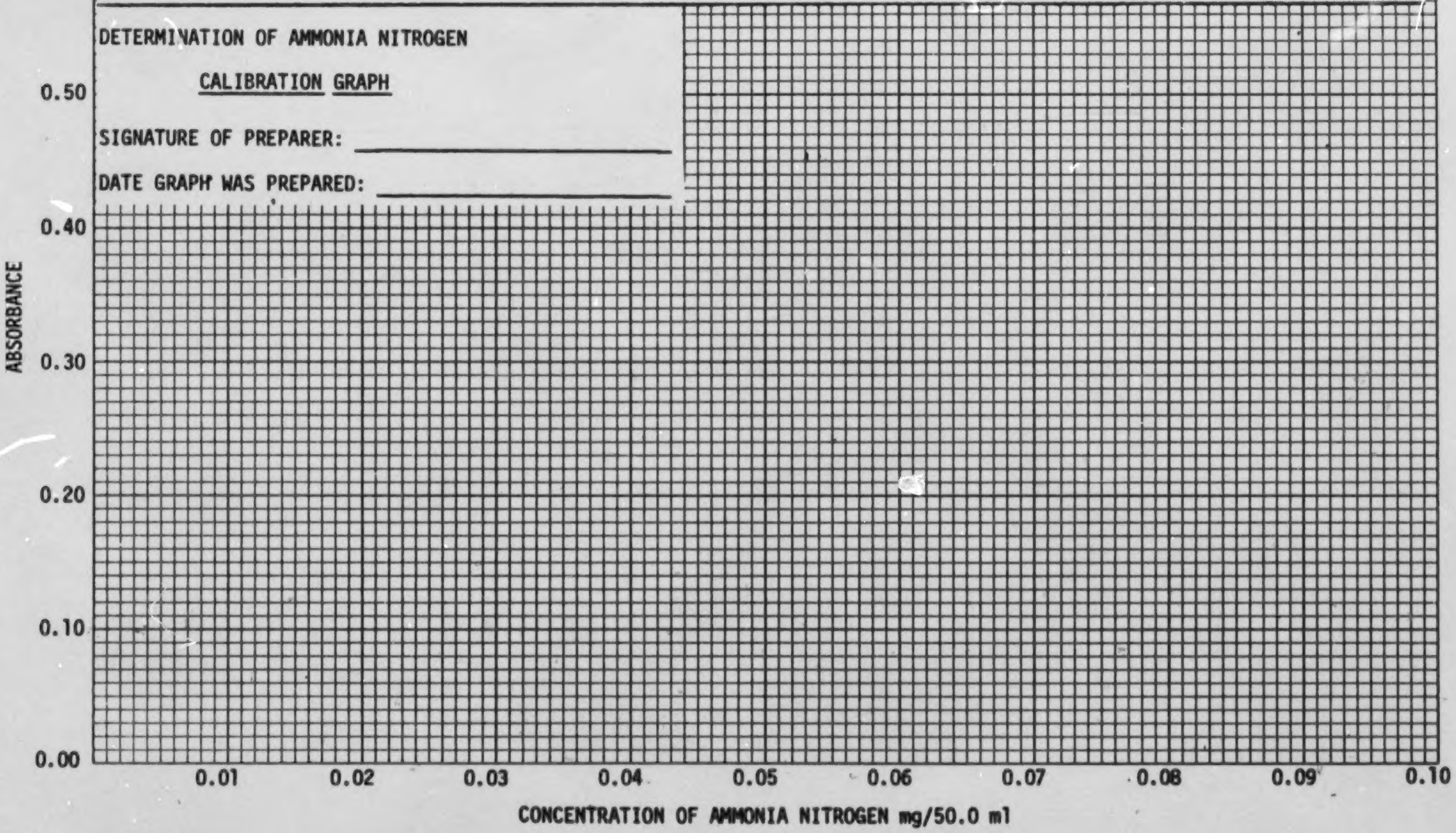
Tube #	Concentration mg NH_3-N/50.0 ml	Absorbance	Absorbance	Absorbance
1	0.0			
2	0.005			
3	0.010			
4	0.020			
5	0.040			
6	0.050			
7	0.080			
8	0.10			
9	Sample			
10	Sample			
11	Sample			

DETERMINATION OF AMMONIA NITROGEN

CALIBRATION GRAPH

SIGNATURE OF PREPARER: ____________________

DATE GRAPH WAS PREPARED: ____________________



A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

NITROGEN, AMMONIA DETERMINATION

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.N.am.EMP.1a.9.75

EFFLUENT MONITORING PROCEDURE: Nitrogen, Ammonia Determination

This operational procedure was developed by:

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ADDRESS	EPA-WPO-National Training Center, Cincinnati, Ohio
POSITION	Chemist Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.S. Chemistry

14 years Industrial Chemist

16 Years HEW-FWPCA-EPA-Chemist

EFFLUENT MONITORING PROCEDURE: Nitrogen, Ammonia Determination

1. Objective:

To determine the nitrogen (as ammonia) content of an effluent

2. Brief Description of Analysis:

The sample is buffered at a pH of 9.5 with a borate buffer and is then distilled into a solution of boric acid. For samples containing ammonia concentration of less than one milligram per liter, the ammonia concentration can be determined colorimetrically. For samples containing higher concentrations (1.0 to 25 mg/liter) the ammonia concentration is determined by a volumetric titration procedure.

3. Applicability of this Procedure:

a. Range of Concentration:

Colorimetric Method - 0.03 to 1.0 mg NH_3-N/liter

Titrimetric Method - 1.0 to 25 mg NH_3-N/liter

(The range of these methods may be extended for samples by dilution.)

NOTE: A range from 0.05 to 1400 mg NH_3-N/liter is available by using an ammonia selective ion electrode. A separate EMP on this method is available.

b. Pretreatment of Samples:

This procedure includes the manual distillation of the sample at pH 9.5 as specified in the Federal Register Guidelines.

c. Treatment of Interferences in Samples:

This procedure includes addition of sodium thiosulfate to remove residual chlorine. If samples contain volatile alkaline compounds or mercury salts (sometimes used as preservatives), consult the Source of Procedure* for appropriate treatments.

*Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, Ohio, p. 159

EFFLUENT MONITORING PROCEDURE: Nitrogen, Ammonia Determination

Equipment and Supply Requirements

A. Capital Equipment

1. Analytical balance, 200 g capacity
2. Trip balance, 500 g capacity
3. Meter, pH
4. Spectrophotometer and cuvettes

B. Reusable

1. Burner, Meker type, gas
2. Safety glasses
3. Laboratory apron
4. Pipettes, volumetric, 1, 2, 5, 25, 50 ml
5. Graduated cylinder, 100 ml
6. Kjeldahl flask, 800 ml
7. Condenser, Allihn, 600 ml
8. Kjeldahl spray trap
9. Support, tripod base, 10 x 24 inch
10. Clamps, two, utility
11. Beaker, 600 ml
12. Flask, Erlenmeyer, 500 ml
13. Reagent bottles, 200 ml, 500 ml
14. Plastic squeeze bottle, 500 ml
15. Nessler tubes, 50 ml

C. Consumable

1. Concentrated sulfuric acid
2. Boiling chips
3. Boric acid
4. Methyl red indicator
5. Ethyl alcohol or denatured (3A or 30)
6. Methylene blue
7. Mercuric iodide
8. Potassium iodide
9. Sodium tetra borate
10. Sodium thiosulfate
11. Sodium hydroxide
12. Ammonium chloride

All reagents should be high quality.

EFFLUENT MONITORING PROCEDURE: Nitrogen, Ammonia Determination

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
NITROGEN, AMMONIA DETERMINATION			I (p. 17)
A. Sample Preservation			
1. Addition of preservative	1. Add 2 ml of concentrated sulfuric acid (H_2SO_4) or 40 mg of mercuric chloride ($HgCl_2$) per liter and store at 4° centigrade.	1a. Because organic nitrogen is progressively ammonified by biologic activity, the determination of ammonia is best made on a fresh sample. 1b. The use of mercuric chloride is discouraged.	I.A.1a. (p. 17)
B. Equipment Preparation			
1. Glassware wash-up	1. Clean all glassware in suitable detergent.	1a. Distilled water drains without leaving any droplets.	
2. Still Cleaning	1. Add 500 ml of ammonia-free water to an 800 ml Kjeldahl flask.	1a. Use deionized distilled water. Shake 4 liters of distilled water with 10 grams of Ionac C-101 cation exchange resin, available from Ionac Chemical Company, Birmingham, NJ*.	
	2. Add a few boiling chips.	2a. The addition of boiling chips which have been previously treated with dilute sodium hydroxide will prevent bumping.	
	3. Set up the still assembly.	3a. Assembly consists of gas burner, distillation flask, condenser and receiving flask.	
	4. Ignite the burner under the flask and apply heat cautiously so that the water boils slowly.		
	5. Test the distillate by adding about 5.0 ml of Nessler's reagent.	5a. If the distillate remains colorless, the glassware is not contaminated with ammonia.	

*Cation exchange resins are available from many manufacturers. This recommendation is not an endorsement this particular product.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation			
1. Boric Acid Solution	1. Dissolve 20 grams of boric acid (H_3BO_3) in distilled water and dilute to one liter with distilled water.	1a. This is a 2 percent solution of boric acid.	
2. Mixed Indicator Solution	1. Dissolve 200 mg methyl red indicator in 100 ml 95% ethyl alcohol.	1a. Specially denatured ethyl alcohol conforming to formula 3A or 30 of the U.S. Bureau of Internal Revenue may be substituted for 95% ethanol.	
	2. Dissolve 100 mg methylene blue in 50 ml of 95% ethyl alcohol.		
	3. Transfer the above two solutions into a dispensing glass bottle.	3a. This solution should be prepared fresh every 30 days.	
3. Nessler Reagent	1. Dissolve 100 grams of mercuric iodide and 70 grams of potassium iodide in about 300 ml of distilled water.	1a. Mercuric iodide dissolves after potassium iodide is added.	
	2. Add the above mixture slowly to a cooled solution of 160 grams of sodium hydroxide previously dissolved in 500 ml of distilled water.	2a. Use a glass rod for stirring or a magnetic stirrer when the mixture is being added.	
	3. Dilute the mixture to 1 liter.	3a. Store the reagent in a pyrex glass bottle. Keep out of direct sunlight. It will remain stable for a period of up to one year.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Borate Buffer	1. Add 10 ml 1 N sodium hydroxide to 50 ml of distilled water and dilute to the mark with distilled water.	1a. Use a 100 ml volumetric flask. 1b. This solution will have a concentration of 0.1 N. 1c. See reagent #6 for 1 N sodium hydroxide preparation.	
	2. Add 4.75 grams of sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) to about 300 ml distilled water in a 500 ml volumetric flask.		
	3. Dissolve and dilute to the 500 ml volume with distilled water.	3a. This solution will have a concentration of 0.025 M	
	4. Add 88 ml of the 0.1 N NaOH (Step 1) to a 1 liter flask.		
	5. To the same flask add 500 ml of the 0.025 M sodium tetraborate (Step 3).		
	6. Swirl to mix and dilute to the 1 liter volume with distilled water.	6a. This is the borate buffer solution.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
5. Sulfuric Acid Stock Solution Approximately 0.1N	1. Add 3 ml of concentrated sulfuric acid (specific gravity 1.84) to about 800 ml of CO_2 free distilled water. Mix well and dilute to 1000 ml with CO_2 free distilled water.	1a. Use a 3 ml pipette. A pipette bulb must be used. 1b. Use a 1000 ml volumetric flask.	
	2. Dilute 200 ml of this solution to one liter with CO_2 free distilled water.	2a. Use a 1000 ml volumetric flask. The concentration of this solution should be about 0.02N. 2b. Standardize according to the procedure prescribed in the EMP "Determination of Total Kjeldahl Nitrogen."	
6. Sodium Hydroxide 1N	1. Dissolve 40 grams of sodium hydroxide (NaOH) in ammonia-free water and dilute to one liter.	1a. Use a volumetric flask. 1b. Transfer reagent to pyrex reagent bottle fitted with a rubber stopper.	
7. Sodium Thiosulfate (1/70N)	1. Dissolve 3.5 grams of sodium thiosulfate pentahydrate in about 300 ml of distilled water and dilute to one liter with distilled water.	1a. This solution can be used to remove residual chlorine from the sample prior to distillation. 1b. One ml of this solution will remove 1 mg/liter of residual chlorine in 500 ml of sample. 1c. Use sodium thiosulfate pentahydrate $Na_2S_2O_3 \cdot 5H_2O$.	
8. Stock ammonium chloride (1 ml = 1.0 mg of ammonia nitrogen)	1. Dissolve 3.819 grams of NH_4Cl in water and dilute to 1 liter.	1a. Wherever water is mentioned it refers to ammonia-free water.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
9. Working ammonium chloride solution (1 ml = 0.01 mg of ammonia nitrogen)	1. Dilute 10.0 ml of the NH_4Cl to 1 liter with water.	1a. Use the stock ammonium chloride solution (reagent #8).	
D. Procedure (Sample contains 1.0 to 25.0 mg/l ammonia nitrogen)	1. Add 500 ml of ammonia-free water to an 800 ml Kjeldahl flask.	1a. Use a graduate cylinder.	
	2. Add a few boiling chips to the flask.	2a. The addition of boiling chips which have been previously treated with dilute sodium hydroxide will prevent bumping during the distillation process.	
	3. Set up the still assembly as before.	3a. Cooling water to condenser turned off.	
	4. Ignite the burner and steam out the distillation apparatus.	4a. Periodically check the distillate in the receiving flask by adding a few milliliters of Nessler's reagent. If the distillate remains colorless, the apparatus is not contaminated with any trace of ammonia.	
	5. Continue the cleaning process until you are assured that no traces of ammonia are present.		
	6. Transfer a 400 ml aliquot of sample into a 600 ml beeker..	6a. If chlorine is present in the sample it must be removed prior to the distillation by adding 1 ml of sodium thiosulfate for each 1 mg/liter of residual chlorine in 500 ml of sample.	VII.D.6a. (p. 18)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	7. Add sodium hydroxide solution (1N) until the pH is raised to 9.5.	7a. Use a magnetic stirrer or a glass stirring rod for stirring. Use a dropping bottle for the addition of sodium hydroxide. 7b. Check the pH during the addition with the use of a pH meter or by the use of short range pH paper.	VII.D.7a. (p. 18)
	8. Transfer the sample to the previously steam-cleaned 800 ml Kjeldahl flask.		
	9. Add 25 ml of the borate buffer.		
	10. Attach the flask and connect the still assembly.	10a. Turn on water to cooling condenser.	
	11. Add 50 ml of 2 percent boric acid to the 500 ml receiving flask, and position the flask under the condenser tip.	11a. The condenser tip should be adjusted so that it is below the surface of the liquid.	
	12. Ignite the burner and distill 300 ml at the rate of 6 to 10 ml per minute.		
	13. Remove the receiving flask and turn off the burner.		
	14. Add 3 drops of mixed indicator to the receiving flask and its contents.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	15. Set up a titration burette and fill it with 0.02 N sulfuric acid standard solution. 16. The color change at the end point in the titration should match the color change produced at the end point when a plain distilled water sample is run through the same procedure using all reagents that would be used for a sample.	15a. Use a 50 ml burette.	
E. Calculations for Titration Procedure (Sample contains 1.0 to 25.0 mg/l ammonia nitrogen)	1. The amount of ammonia nitrogen present can be determined with the use of the formula to the right. 2. There is a calculation sheet page 19.	1a. NH_3-N mg/l = $\frac{A \times 0.28 \times 1000}{S}$ where: A - will equal the milliliters of 0.02 N sulfuric acid used in the titration S - will equal the milliliters of sample used in the test An example of the use of this formula for the analysis of a wastewater sample follows: 1. Sample size for the analysis = 400 ml 2. ml of 0.02 N sulfuric acid = 17.0 3. NH_3N mg/l = $\frac{17.0 \times 0.28 \times 1000}{400}$ NH_3N mg/l = 11.9 mg/l	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	3. If the sample contains more than 25 mg/liter of ammonia, an appropriately smaller sample size must be used.		
	4. If the sample contains 1.0 mg or less ammonia nitrogen per liter, the following procedure should be used.		
F. Procedure (Sample contains 0.05 to 1.0 mg ammonia nitrogen per liter)	1. Transfer a 400 ml sample into a 600 ml beaker.		
	2. Add 1N NaOH with an eye dropper until the pH is 9.5.	2a. Use a pH meter or pH paper and stir the solution during the addition of sodium hydroxide.	
	3. Transfer the pH 9.5 sample to a steam cleaned 800 ml Kjeldahl flask and add 25 ml of borate buffer.	3a. Use a 50 ml graduate for the buffer addition.	
	4. Distill 300 ml at the rate of 6-10 ml/minute into 50 ml of 2 percent boric acid contained in a 500 ml glass stoppered Erlenmeyer flask.		
	5. Dilute to 500 ml.	5a. Use ammonia-free water.	
	6. Into 50 ml Nessler tubes pipet the following volumes of the working ammonium chloride solution: 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 8.0, and 10.0 ml.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	7. Add sufficient ammonia free water to bring the volume to 50 ml.		
	8. Pipet into three Nessler tubes 2, 25, and 50 ml of the distilled sample from step 5 above. Dilute with water to 50 ml.	8a. Use a volumetric pipette. The purpose of using three aliquots is to ensure that when the colors are developed, one of the three will produce a color which lies within the range of the calibration curve.	
	9. Pipette 1.0 ml of the Nessler reagents into each standard and sample tube and mix.	9a. Use a volumetric pipette.	
	10. Transfer appropriate aliquots into cuvettes for measurement of the color intensity in the spectrophotometer.		
	11. Read the absorbance of all tubes after 20 minutes at 425 nanometers against the the 0.0 standard.	11a. There is an EMP on "Use of a Spectrophotometer."	
G. Calculations for Colorimetric Procedure (Sample contains 0.05 to 1.0 mg ammonia nitrogen per liter)	1. Prepare a calibration curve of absorbance values of the standards versus mg of ammonia nitrogen. For example: if 2.0 ml of the working NH_4Cl are used, and its concentration is 0.01 mg of NH_3-N/ml, then 0.02 mg is the value plotted on the calibration curve versus the corresponding absorbance.	1a. There is an EMP on "Preparation of Calibration Graphs."	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	2. Determine the amount of NH_3-N present in the sample from the calibration curve.		
	3. Determine the mg of NH_3-N/liter of sample using the formula: $\text{mg/l } NH_3\text{-N} = \frac{A \times 1000}{D} \times \frac{B}{C}$	3a. A = mg NH_3-N read from standard curve B = ml total distillate collected, including boric acid and dilution C = ml distillate taken for nesslerization D = ml of original sample taken An example calculation using a value from a calibration curve would be: A = 0.015 mg (read from standard curve) B = 500 ml (300 ml distillate + 50 ml boric acid + 150 ml dilution water) C = 25 ml (distillate which was diluted for nesslerization) D = 400 ml (ml of original sample taken) $\text{mg/l } NH_3\text{-N} = \frac{A \times 1000}{D} \times \frac{B}{C}$ $= \frac{0.015 \times \overset{50}{1\cancel{000}}}{\underset{\underset{1}{\cancel{20}}}{4\cancel{00}}} \times \frac{\overset{\overset{1}{\cancel{20}}}{5\cancel{00}}}{\underset{1}{\cancel{25}}}$ $= 0.015 \times 50$ $\text{mg/l } NH_3\text{-N} = 0.75$ There is a calculation sheet on page 20.	

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

INTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1a.	The compounds of nitrogen are of interest because of the importance of nitrogen in the life processes of all plants and animals. Chemists analyzing sewage and freshly polluted waters learned that most of the nitrogen is originally present in the form of organic (protein) nitrogen and ammonia. As time progresses, the organic nitrogen is gradually converted to ammonia nitrogen, and later on, if aerobic conditions are present, oxidation of ammonia to nitrites and nitrates occurs. Waters that contain mostly organic and ammonia nitrogen are considered to be recently polluted and therefore of great potential danger. Waters in which most of the nitrogen is in the form of nitrates are considered to have been polluted a long time previously and therefore are not dangerous to the public health. Since the treatment plant is an accelerated version of the natural process of converting nitrogen from one compound to another, the monitoring of the ammonia concentration is an effective means of determining the efficiency of the biodegradation.	Sawyer, C. N. and McCarty, P. L. Chem. for San. Eng., 2nd Ed., McGraw-Hill, 1967
	The test described in this instruction can be found in the 1974 EPA Methods Manual on page 159, entitled Nitrogen, Ammonia (Distillation Procedure). If the distillation is done at pH 9.5, another reference which contains an acceptable procedure for this test is on page 410 of the 14th edition of Standard Methods.	Methods for Chemical Analysis of Water and Wastes, 1974, EPA, MDQARL, Cincinnati, Ohio 45268, p. 159. Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, New York, p. 410

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
VII.D.6a.	At some water treatment plants ammonia is added in the combined residual chlorination of water. Where the free residual chlorination process is employed, ammonia nitrogen will react with chlorine in ratios which vary with the nitrogen concentration. At low ammonia concentrations (0.1 mg/liter nitrogen) the ratio approximates 1 to 10, while at higher ammonia concentrations the ratio approaches 1 part of ammonia nitrogen to 7.59 parts of chlorine. If a sample contains residual chlorine, then monochloramine, dichloramine, or trichloramine may be present. Dechlorination prior to analysis will convert these substances to ammonia.	Standard Methods 13th Ed., p. 223
VII.D.7a.	Ammonia recovery from preliminary distillation will be low on water samples containing more than 250 mg/liter calcium unless the pH is properly adjusted before distillation is undertaken. The calcium and the phosphate buffer react to precipitate calcium phosphate, releasing hydrogen ions and lowering the pH.	Standard Methods 13th Ed., p. 223

LABORATORY DATA SHEET

Nitrogen, Ammonia Determination
(Sample contains 1.0 to 25.0 mg/l NH_3-N)

Sample No. __________ Date/Time Sampled ________________________ Sample Point ________

$$\frac{(\text{Sulfuric acid 0.02N ml \_\_\_\_\_\_\_})(0.28)(1000)}{\text{Sample ml \_\_\_\_\_}} = \text{\_\_\_\_ mg/liter } NH_3\text{-N}$$

_____________________________ Date ______________
Analyst

LABORATORY DATA SHEET

Nitrogen, Ammonia Determination
(Sample Contains 0.05 to 1.0 mg/liter NH_3-N)

Sample No. _________ Date/Time Sampled ______________________ Sample Point _________

$$\frac{(\text{mg of } NH_3\text{-N}\underline{\hspace{1cm}})(1000)}{(\text{Sample ml}\underline{\hspace{1cm}})} \times \frac{(\text{Total Distillate* Collected ml}\underline{\hspace{1cm}})}{(\text{Distillate Taken for Nesslerization ml}\underline{\hspace{1cm}})} = \underline{\hspace{1cm}}\text{mg/l } NH_3\text{-N}$$

______________________________ Date ___________
Analyst

*Include boric acid plus dilution water

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF NITRATE-NITRITE NITROGEN AND OF NITRATE NITROGEN, CADMIUM REDUCTION METHOD

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.N.n/n.EMP.1a.3.76

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

This operational procedure was developed by:

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Miami, Florida 33176

POSITION Chairman - Chemistry Department

EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

M.S. - Chemistry

PhD. - Analytical Biochemistry

1 year Commercial Laboratory Chemist

10 years College Chemistry Instructor

7 years Chemical Consultant to Industry

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

1. Objective:

To determine the nitrate-nitrite nitrogen and the nitrate nitrogen content of an effluent.

2. Brief Description of Analysis:

The procedure converts nitrate nitrogen to nitrite nitrogen when the nitrate is passed through a column containing copper-cadmium granules. Nitrate is almost quantitatively reduced to nitrite by this process. The resulting nitrite is determined by reacting the effluent with sulfanilamide and coupling with N - (1-napthyl) - ethylenediamine dihydrochloride to form a highly colored dye which can then be determined colorimetrically. A correction must be made for any nitrite initially present in the sample since the method determines total nitrite. The concentration of nitrite originally present in a sample can be determined by omitting the initial copper-cadmium reduction and carrying out the remainder of the procedure. Separate nitrate-nitrite values for a sample may be obtained by analyzing two aliquots of the same; one with the copper-cadmium reduction step and one without the initial reduction step.

3. Applicability of this Procedure:

a. Range of Concentration:

0.01 to 1.0 mg NO_3-NO_2 N/liter
(The range may be extended for samples by dilution.)

b. Pretreatment of Samples:

The Federal Register Guidelines do not specify any pretreatment.

c. Treatment of Interferences in Samples:

This procedure includes directions for removal of turbidity and/or of grease and oil from samples. It also includes addition of EDTA to eliminate interferences from metals. No other interferences are noted in the Source of Procedure.*

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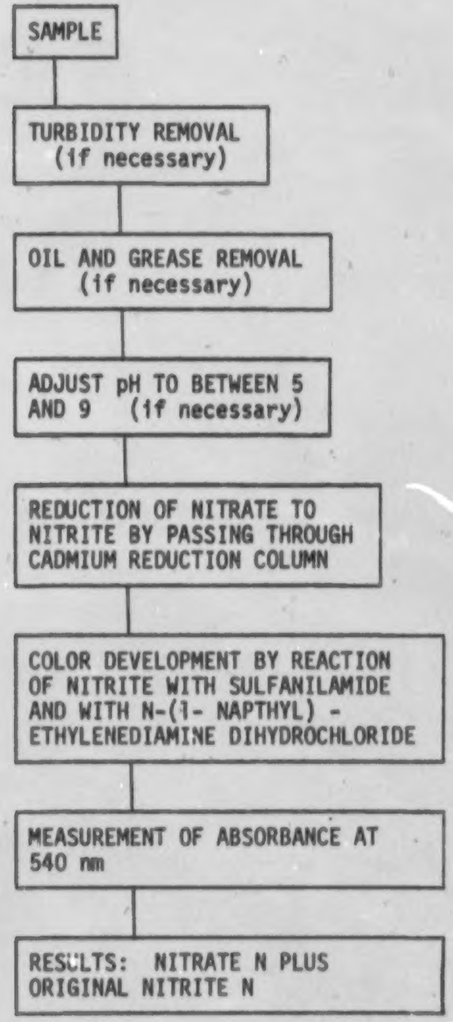
*Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, Ohio, page 201.

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

FLOW SHEET:


SAMPLE
TURBIDITY REMOVAL (if necessary)
OIL AND GREASE REMOVAL (if necessary)
ADJUST pH TO BETWEEN 5 AND 9 (if necessary)
REDUCTION OF NITRATE TO NITRITE BY PASSING THROUGH CADMIUM REDUCTION COLUMN
COLOR DEVELOPMENT BY REACTION OF NITRITE WITH SULFANILAMIDE AND WITH N-(1- NAPTHYL) - ETHYLENEDIAMINE DIHYDROCHLORIDE
MEASUREMENT OF ABSORBANCE AT 540 nm
RESULTS: NITRATE N PLUS ORIGINAL NITRITE N

The above procedures determine nitrate N plus nitrite N. The initial nitrite concentration of the samples should be determined prior to reduction. Thus, the nitrate concentration can be determined by:

Nitrate N = Total Nitrite N - Nitrite N before reduction

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Balance, analytical, 160 g capacity, precision ± 0.1 mg
2. Balance, triple beam, 500 g capacity, precision ± 0.25 g
3. pH meter/combination electrode, range 0-14 pH
4. Refrigerator, temperature range 2° - 10°C
5. Spectrophotometer, wave length range 325-825 nm
6. Still and de-ionizing cartridges (or other means of distilling and de-ionizing water)

B. Reusable Supplies:

1. One apron, laboratory
2. One 100 ml beaker
3. Four 250 ml beakers (3 for buffer solutions)
4. One 400 ml beaker
5. One 1 liter beaker
6. One 2 liter beaker
7. Two bottles, Barnes with stoppers and two droppers, small gauge
8. One 150 ml bottle, dropper
9. One 250 ml bottle, plastic wash
10. One 100 ml bottle, storage with screw-on cap (storage of 6N HCl)
11. Seven 1 liter bottles, storage, brown with screw-on caps or rubber stoppers
12. Two 5 gallon bottles, water with bottom spout
13. One brush, camel hair (cleaning analytical balance)
14. Two brushes, bottle (cleaning glassware)
15. One bulb, propipet type
16. One buret holder, double clamps (reduction column support)
17. Two columns, reduction (see Figure 1 at the end of this section)
18. Three cuvettes
19. One 25 ml cylinder, graduated
20. One 50 ml cylinder, graduated
21. One 100 ml cylinder, graduated
22. One 500 ml cylinder, graduated
23. One 1 liter cylinder, graduated
24. One 50 ml flask, volumetric with stopper (dilution of sample)
25. Twelve 100 ml flasks, volumetric with stoppers (for standards)
26. X 100 ml flasks, volumetric with stoppers (for samples - 1 flask per sample)
27. Twelve 250 ml flasks, Erlenmeyer with stoppers (for standards)
28. X 250 ml flasks, Erlenmeyer with stoppers (for samples-1 flask per sample)
29. One 1 liter flask, Erlenmeyer, or a large, empty chemical bottle (for Cd washings)
30. Three 1 liter flasks, volumetric with stoppers
31. Two 2 liter flasks, volumetric with stoppers
32. One filter funnel for 0.45 μ filter (turbidity removal)

B. Reusable Supplies (Continued)

33. One funnel, powder
34. One funnel, large powder with large filter paper (for Cd washings)
35. One 250 ml funnel, separatory (oil and grease removal)
36. One pair glasses, safety
37. Two hoses, rubber, 3" strip, 4 cm I.D. with screw type clamp
38. One notebook (recording data)
39. Two 100 ml volumetric pipets (construction of reduction columns)
40. One 0.5 ml pipet, volumetric
41. One 1 ml pipet, volumetric
42. One 2 ml pipet, volumetric
43. One 5 ml pipet, volumetric
44. One 10 ml pipet, volumetric
45. One 25 ml pipet, volumetric
46. One 50 ml pipet, volumetric
47. One rod, stirring (6" or 12")
48. One sieve, 40 mesh
49. One sieve, 60 mesh
50. One spatula (scoopula)
51. Two stands, ring (support funnel, and reduction column)
52. One support, ring, small (support funnel)

C. Consumable Supplies:

1. Glasswool, wad
2. Membrane filter, 0.45 μ
3. Notebook (recording data)
4. Pen or pencil (recording data, marking flasks)
5. Soap
6. Sponges (for cleaning)
7. Tissues, soft (wiping cuvettes and electrodes)
8. Towels, paper
9. Twelve weighing boats
10. 26 g ammonium chloride, NH_4Cl

*11. 100 ml ammonium hydroxide, NH_4OH

*12. 150 ml buffer solution, STD pH 4
*13. 600 ml buffer solution, STD pH 7
*14. 450 ml buffer solution, STD pH 10
**15. 25 g cadmium granules, 40-60 mesh
16. 55 ml chloroform, $CHCl_3$

17. 20 g copper sulfate, pentahydrate, $CuSO_4 \cdot 5H_2O$

18. 3.4 g disodium ethylenediamine tetraacetate, $C_{10}H_{14}N_2Na_2O_8$

19. 1 g N-(1-napthyl) - ethylenediamine dihydrochloride, $C_{12}H_{14}N_2 \cdot 2HCl$

*20. 200 ml hydrochloric acid, concentrated, HCl
21. 100 ml hydrochloric acid, dilute (6N), HCl
22. 100 ml phosphoric acid, concentrated, H_3PO_4

*23. Potassium dichromate (cleaning solution), $K_2Cr_2O_7$

24. 7.218 g potassium nitrate, KNO_3

C. Consumable Supplies (Continued)

25. 6.072 g potassium nitrite, KNO_2
26. 240 g sodium hydroxide, pellets, NaOH
27. 10 g sulfanilamide, $C_6H_8N_2O_2S$

*28. Sulfuric acid, concentrated, (cleaning solution) H_2SO_4

29. 100 g zinc sulfate, heptahydrate, $ZnSO_4 \cdot 7H_2O$
30. Labels, package, 1 1/2 x 1 inch
31. Paper, graph 8 1/2 x 11, package

All reagents should be reagent grade.

The above amounts do not allow for spillage or mistakes.

*These amounts will vary

**This metal can be purchased from EM Laboratories, Inc., 500 Executive Boulevard, Elmsford, New York, 10523, Cat. 2001 cadmium, coarse powder

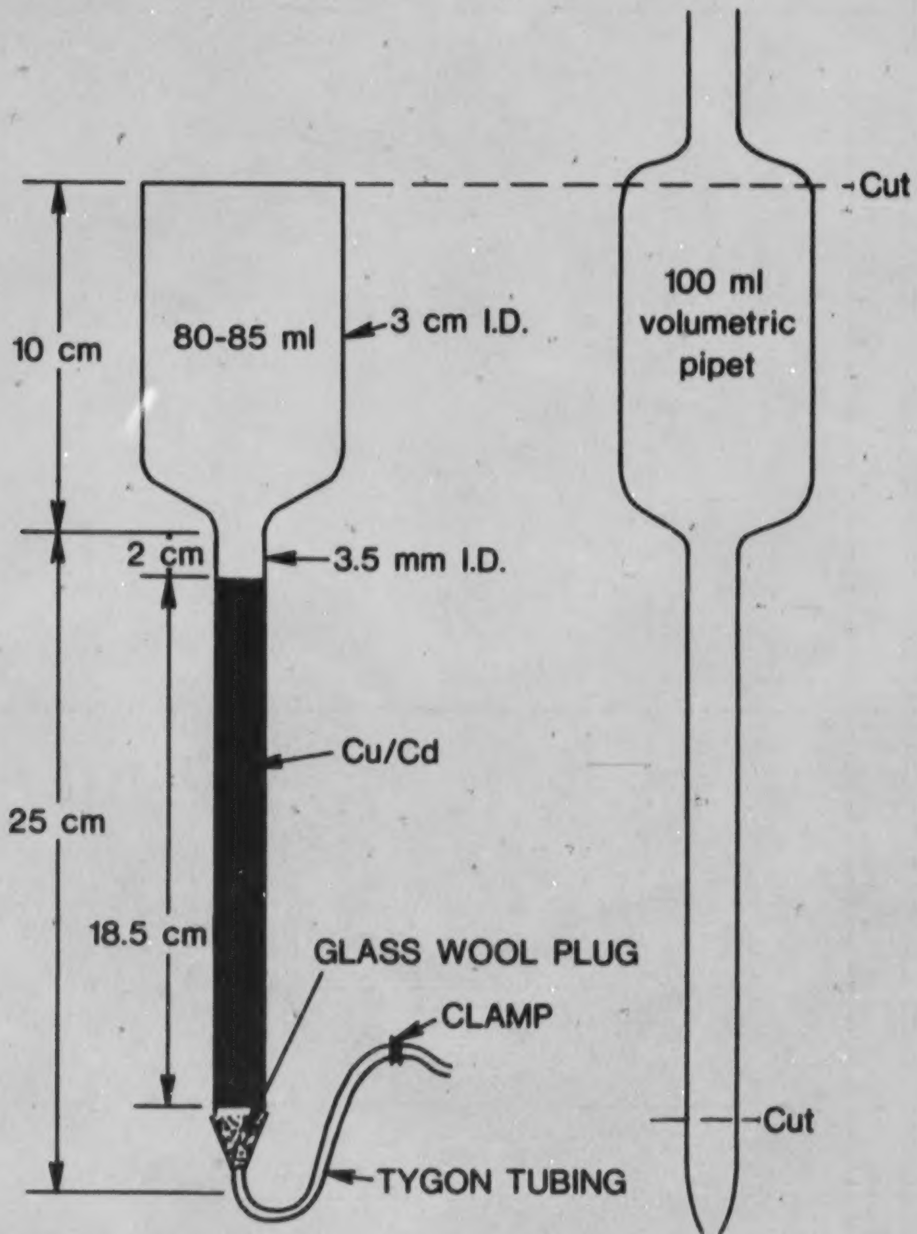

Cut
100 ml volumetric pipet
10 cm
80-85 ml
3 cm I.D.
2 cm
3.5 mm I.D.
Cu/Cd
25 cm
18.5 cm
GLASS WOOL PLUG
CLAMP
Cut
TYGON TUBING

Figure 1. Reduction column

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
DETERMINATION OF NITRATE-NITRITE NITROGEN AND OF NITRATE NITROGEN, mg/liter			I (p. 41)
A. Equipment Preparation			
1. Glassware Wash-Up	1. Clean all glassware in suitable detergent.	1a. Distilled water drains without leaving any droplets on surfaces. 1b. Use chromerge if necessary.	
2. Balance Inspection	1. Clean balance.	1a. Free of dust and dirt.	
3. Spectrophotometer Inspection	1. Clean spectrophotometer.	1a. Free of dust and dirt.	
	2. Turn power on by rotating the power control clockwise.	2a. Pilot lamp on. 2b. Directions are for Spectronic 20.	
	3. Select wavelength by rotating the wavelength control knob either direction until the proper wavelength is reached.	3a. 540 nm on the wavelength scale.	
	4. Zero the instrument by bringing the meter needle to "0" on the percent transmittance scale.	4a. Meter needle reads zero.	
	5. Use an empty cell and adjust the light control to 100% T.	5a. To be sure that the instrument can achieve 100% T.	

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation			
1. Distilled Water	1. Prepare approximately ten (10) liters of highly pure water.	1a. An ion exchange column in conjunction with a still provides an adequate source of highly pure water. 1b. This water will be used for all reagent preparation and washing of equipment. 1c. The pH of the water must be between 5.5-7.5.	
2. Concentrated Ammonium Chloride EDTA Solution	1. Weigh 26 g of ammonium chloride, NH_4Cl, in a weighing boat and wash into 2.0 liter graduated beaker.	1a. Distilled water should be used for all phases of solution preparation including water used in washing a solid into a container.	
	2. Weigh 3.4 g of disodium ethylenediamine tetraacetate, $C_{10}H_{14}N_2Na_2O_8$, and wash into the same beaker.		
	3. Add enough distilled water to bring the total volume to approximately 1800 ml.		
	4. Use a pH meter to adjust the pH of the solution to 8.5 by the dropwise addition of concentrated ammonium hydroxide, NH_4OH.	4a. Mix the solution thoroughly by stirring, after the addition of each drop of NH_4OH.	
	5. After the pH has been adjusted, transfer the solution to a 2 liter volumetric flask.	5a. Whenever a solution is transferred, the container from which the transfer is made should be washed and the washings added to the container to which the transfer was made.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)			
	6. Dilute to volume with distilled water.	6a. The solution is stable for several months.	
	7. Label the bottle in which the solution is stored.	7a. Include the name of the solution, your name and the date of preparation.	
3. Dilute Ammonium Chloride EDTA Solution	1. Measure 1200 ml of the concentrated ammonium chloride-EDTA solution, using a graduated cylinder.		
	2. Pour the measured solution into a 2.0 liter volumetric flask.		
	3. Dilute to volume with distilled water.		
	4. Store in a labeled container.	4a. Both the concentrated and dilute ammonium chloride-EDTA solutions are stable for several months.	
4. Color Reagent	1. Add 800 ml of distilled water to a 1 liter flask.	1a. Use a graduated cylinder. 1b. Use a 1 liter volumetric flask.	
	2. Add 100 ml of concentrated phosphoric acid, H_3PO_4, to the same flask.		
	3. Mix thoroughly.		
	4. Weigh 10 g of sulfanilamide ($C_6H_8N_2O_2S$) in a weighing boat.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	5. Use a wash bottle and funnel to wash the sulfanilamide into the 1 liter flask containing phosphoric acid solution.		
	6. Weigh 1 g N-(1-napthyl)-ethylenediamine dihydrochloride, Marshall's Reagent, and wash into same flask.		
	7. Dilute to volume with distilled water.		
	8. Store in a labeled container.	8a. Container should be dark 1 liter plastic reagent bottle. 8b. Store at 4°C when not in use. 8c. Use at room temperature. 8d. The solution is stable for several months. 8e. A very faint pink color may show up in this color reagent. You may still use the reagent. If a precipitate forms in the reagent, though, discard it.	
5. Zinc Sulfate Solution	1. Weigh 100 g of zinc sulfate heptahydrate, $ZnSO_4 \cdot 7H_2O$, in a weighing boat.	1a. This reagent is used if flocculation is employed as an alternative to filtration if the sample requires removal of turbidity.	
	2. Wash into a 1 liter flask using a wash bottle and a funnel.	2a. Use a volumetric flask.	
	3. Add sufficient distilled water to dissolve all of the solid.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	4. Dilute to volume with distilled water.		
	5. Store in a labeled container.	5a. This solution is stable for at least one year.	
6. Sodium Hydroxide Solution (6N)	1. Rapidly weigh 240 g of solid sodium hydroxide, NaOH, pellets in a 1 liter graduated beaker.	1a. This reagent is used if flocculation is employed as an alternative to filtration if the sample requires removal of turbidity. 1b. Sodium hydroxide picks up moisture from the air quite readily.	
	2. Add 500 ml distilled water to dissolve the sodium hydroxide.	2a. The water should be added with constant swirling to avoid fusing.	
	3. Dilute to a total volume of 1 liter.	3a. The solution should be allowed to cool to room temperature before the dilution is made.	
	4. Store in a glass bottle or jug and stopper with a rubber stopper.	4a. Sodium hydroxide slowly etches glass causing glass stoppers to stick. 4b. The solution is stable for at least a year.	
	5. Label the container.		
7. Ammonium Hydroxide	1. A 100 ml supply should be available.	1a. Drop quantities may be required for pH adjustment.	
	2. Place in a Barnes (dropper) bottle.		
8. Hydrochloric Acid, (6N)	1. Add 50 ml of distilled water to a 400 ml beaker.	1a. A 100 ml graduated cylinder is suitable for measuring the volume of the distilled water and the volume of the acid.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	2. Slowly add 50 ml of concentrated hydrochloric (HCl) acid (12 N) to the same beaker.		
	3. Mix thoroughly.		
	4. Store in a 100 ml bottle.		
	5. Label the container.		
9. Copper Sulfate Solution (2%)	1. Weigh 20 g of copper sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$, in a weighing boat.		
	2. Wash copper sulfate into a one liter volumetric flask.		
	3. Add sufficient distilled water to dissolve the solid.	3a. About 500 ml of water should be sufficient.	
	4. Dilute to volume with distilled water.	4a. This solution is stable for at least one year.	
	5. Store in a labeled container.		
10. Nitrate Stock Solution	1. Carefully weigh 7.218 g of potassium nitrate, KNO_3, in weighing boat.	1a. An analytical balance should be used.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	2. Transfer the solid to a 1 liter volumetric flask equipped with a powder funnel.	2a. This is best achieved by washing the solid onto the funnel with a wash bottle.	
	3. Use wash bottle to wash the solid into the flask.	3a. The weighing boat should be rinsed three times and all of the rinse water should be added to the flask.	
	4. Add sufficient distilled water to dissolve the solid.	4a. About 500 ml is sufficient.	
	5. Dilute to volume with distilled water and thoroughly mix.		
	6. Store in a labeled glass bottle.		
	7. Preserve the solution by adding 2 ml of chloroform, $CHCl_3$.	7a. The solution prepared, stored and preserved in this manner should be stable for at least 6 months. 7b. The nitrate stock solution contains 1.00 mg of nitrate nitrogen (NO_3-N) in each 1.00 ml of solution.	
11. Nitrate Standard Solution	1. Carefully pipet 10.0 ml of nitrate stock solution into a 1 liter volumetric flask.	1a. This nitrate standard solution should be prepared fresh for each use. 1b. The nitrate stock solution should be at room temperature before using. 1c. Use a 10 ml volumetric pipet.	
	2. Dilute to volume with distilled water.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	3. Store in a labeled container.	3a. Use within one hour of preparation. 3b. The nitrate standard solution contains 0.01 mg of nitrate nitrogen (NO_3-N) in each 1.0 ml of solution.	
12. Nitrite Stock Solution	1. Weigh 6.072 g of potassium nitrite, KNO_2, in a weighing boat.	1a. An analytical balance should be used for all weighings involving standards.	
	2. Transfer the solid to a 1 liter volumetric flask using a powder funnel.		
	3. Use wash bottle to wash the solid into the flask.	3a. The weighing boat should be washed three times and the washings added to the flask.	
	4. Add sufficient distilled water to dissolve the solid.	4a. About 500 ml is sufficient.	
	5. Dilute to volume and mix thoroughly.		
	6. Store in a labeled glass bottle.		
	7. Preserve the solution by adding 2 ml of chloroform for each 1 liter of solution and refrigerate when not in use.	7a. The solution should be stable for at least 3 months when preserved this way and stored at about 4°C when not in use. 7b. The nitrite stock solution contains 1.00 mg of nitrite nitrogen (NO_2-N) in each 1.0 ml of solution.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)			
13. Nitrite Standard Solution	1. Pipet 10.0 ml of nitrite stock solution into a 1 liter volumetric flask.	1a. This nitrite standard solution should be prepared fresh for each use. 1b. The nitrite stock solution should be at room temperature before using. 1c. Use a 10 ml volumetric pipet.	
	2. Dilute to volume with distilled water.		
	3. Store in a labeled container.	3a. Use within 1 hour of preparation. 3b. The nitrite standard solution contains 0.01 mg of nitrite nitrogen (NO_2-N) in each 1.0 ml of solution.	
C. Reduction Column Preparation			
1. Preparation of the Glass Column	1. Construct a glass column by joining a 10 cm length of 3 cm ID glass tubing with a 25 cm length of 3.5 mm ID tubing using figure 1 as a guide.	1a. Figure 1 is at the end of the Equipment and Supply Requirements Section. 1b. The column shown in Figure 1 was constructed by cutting both ends off a 100 ml volumetric pipet as indicated. 1c. Fire polish all cut surfaces.	
	2. Loosely plug the delivery tip of the column with glass wool.		

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)			
2. Preparation of Copperized Cadmium for Packing the Glass Column	1. Weigh about 20 g of cadmium granules in a weighing boat.	1a. This will be enough for one column.	
		1b. Granulated cadmium (40-60 mesh) can be purchased.	
		1c. Alternatively, file sticks of pure cadmium metal (reagent grade) with a coarse metal hand file (about second cut) and collect the fraction which passes a sieve with 10 mesh openings and is retained on sieves with 40, then 60 mesh openings.	
		1d. Handling cadmium is hazardous, thus filing should be conducted under a hood using rubber gloves and mask.	VIII.C.2.1d (p. 46)
	2. Transfer the cadmium to a 400 ml beaker.	2a. A scupula and wash bottle with water is good for this.	
	3. Add enough dilute (6N) hydrochloric acid to cover the granules.		
	4. Swirl the contents of the beaker.		
	5. Pour off the acid while retaining the granules in the beaker.	5a. All decanting should be done into a container equipped with a large funnel and filter paper so as to catch all the small cadmium particles.	
		5b. Use this filter paper for any subsequent cadmium washings.	
	6. Add enough distilled water to cover the granules.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)	7. Pour off the water while retaining the granules in the beaker.		
	8. Repeat steps 6 and 7, above, two more times so that the granules receive a total of three distilled water washings.		
	9. Add 100 ml of the 2% copper sulfate solution to the granules and swirl for five minutes or until the blue color of the copper sulfate fades.	9a. A brown colloidal (very fine) precipitate of metallic copper may form.	
	10. Carefully decant off the solution leaving the copperized cadmium granules in beaker.	10a. Also decant off through the filter paper any precipitate that formed.	
	11. Repeat steps 9 and 10 until a brown colloidal (very fine) precipitate of metallic copper does form.		
	12. Wash the copper-cadmium at least 10 times with distilled water.	12a. All of the brown precipitated copper should be removed by washing 10 times but continue to wash if any remains.	
	13. Place the washed copper-cadmium on the 60 mesh sieve.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)	14. Pour water over the granules at least three times so that all the small particles will wash through the 60 mesh screen.	14a. Hold the sieve over the filter paper during these washings.	
	15. Return meshed granules to the beaker.	15a. Use a scupula and the wash bottle.	
	16. Decant off excess water used to transfer the cadmium.		
	17. Close the clamp on the column delivery tube.		
	18. Fill the column almost to the top of the cup part with ammonium chloride-EDTA solution.	18a. Use a graduated cylinder and very slowly pour the solution down the inside wall of this column so air pockets do not form.	
	19. Loosely fill the reduction column with copper cadmium granules to a level about 2 cm below the broad, cup-like section as shown in Figure 1.	19a. Avoid tight packing of granules by allowing the granules to "float" down through the solution of ammonium chloride-EDTA. 19b. A glass stirring rod may be used to transfer the cadmium to the column. 19c. For regeneration of column see training guide. 19d. When column is not in use, fill it with ammonium chloride-EDTA solution so that the granules are covered with about 2.5 cm of solution above them.	VII.C.2.19c (p. 43)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)	20. Open the screw clamp and measure the flow rate of ammonium chloride-EDTA solution through the column.	20a. To calculate the flow rate, place a short 50 ml graduated cylinder under column and measure the amount of fluid collected in one minute. 20b. The flow rate should be between 7 ml and 10 ml/minute. 20c. If the flow rate is too fast, tighten the screw clamp. If the clamp must be so tight that control is lost, add more copper cadmium granules to the column. 20d. If the flow rate is too slow, decrease the length of the copper cadmium column until a flow rate of 7-10 ml/minute is achieved.	
	21. When the flow rate can be maintained between 7 ml and 10 ml/minute, drain off the ammonium chloride-EDTA solution until it is about 2.5 cm above the top of the granules.	21a. When the column is not in use, the copper cadmium granules should be covered with ammonium chloride-EDTA solution so they do not dry out.	
	22. Close the screw clamp.		
D. Removal of Interferences			VI.D (p. 42)
1. Turbidity Removal (If Necessary)	1. Prior to analysis, remove turbidity from samples by filtering through a 0.45 μ membrane filter.	1a. If the turbidity is not removed by filtration, proceed as follows: Add 1 ml of the zinc sulfate solution to 100 ml of sample. Add enough 6 N sodium hydroxide to bring the pH to 10.5, (about 8 to 10 drops is usually sufficient). Let the treated sample stand for 15 minutes. Filter through a 0.45 μ membrane filter. 1b. Suspended solids can clog the reduction column.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Removal of Interferences (Continued)			
2. Oil and Grease Removal (If Necessary)	1. Prior to analysis, measure 100 ml of the sample (filtered sample if the original sample was turbid) into a 400 ml beaker.	1a. Oil and grease can clog the reduction column and coat the Cu/Cd granules.	
	2. By dropwise addition, add sufficient concentrated hydrochloric acid (12 N) to bring the pH down to 2.	2a. Use a pH meter in adjusting the pH to 2. 2b. Standardize using standard buffer of pH = 4.00.	
	3. Place the sample in a 250 ml separatory funnel.		
	4. Add 25 ml of chloroform.		
	5. Shake gently to extract the oils and grease into the chloroform layer.	5a. Carefully release the pressure after shaking gently so that no sample is lost. This can be accomplished by inverting the separatory funnel and slowly opening the stopcock away from face and other people.	
	6. Allow the separatory funnel to stand until all of the chloroform layer settles to the bottom.	6a. Place funnel in ring stand. 6b. Remove stopper while layer is settling.	
	7. Open the stopcock and allow the bottom (chloroform) layer to pass into a 400 ml beaker.	7a. Grease and oils are extracted into chloroform layer leaving a grease-oil free sample which is used for analysis.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Removal of Interferences (Continued)	8. Repeat steps 4, 5, 6, and 7 with 25 ml of fresh chloroform.	8a. The second chloroform extract is added to the same beaker as the first extract.	
E. Preparation of Nitrate Working Standards			
1. Nitrate Working Standards	1. Prepare nitrate working standards by respectively pipetting the following volumes of nitrate standard solution into each of six 100 ml volumetric flasks.	1a. Label flasks. 1b. Use appropriate volumetric pipets (0.5 ml, 1.0 ml, 2.0 ml, 5.0 ml, 10.0 ml). 1c. The 0.00 solution which contains no nitrate (or nitrite) serves as the reagent blank for the nitrate samples and standards which are passed through the reduction column.	

To Flask No.	Add This Volume of Nitrate Standard Solution	For This Concentration of NO_3-N in mg/l
1	0.0 ml	0.00
2	0.5 ml	0.05
3	1.0 ml	0.10
4	2.0 ml	0.20
5	5.0 ml	0.50
6	10.0 ml	1.00

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	2. Dilute each of the flasks to volume with distilled water.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Preparation of Nitrate Working Standards (Continued)	3. Use the working standards immediately after their preparation.	—	
F. Reduction of Nitrate to Nitrite			
1. Adjustment of pH	1. Use a pH meter to adjust the pH of each of the working standards to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.	1a. Use a beaker small enough for this volume of standard to cover the pH electrode(s). 1b. Make sure that the pH meter is calibrated within this range. 1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter. 1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)	
2. Activation of Column	1. Pipet 25.0 ml of working standard #6 to a small Erlenmeyer flask.	1a. Activation of column is necessary to prepare surfaces of Cu-Cd granules for reduction process. 1b. This standard is 1.00 mg NO_3-N/liter concentration. 1c. A 250 ml flask is good for this purpose.	
	2. Add 75 ml of the dilute ammonium chloride-EDTA solution to the same flask.	2a. A 100 ml graduated cylinder is good for this purpose.	
	3. Mix the working standard thoroughly by swirling the contents of the flask.		
	4. Place a 250 ml beaker under the reduction column.	4a. You will collect the reduced working standard in this beaker.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Reduction of Nitrate to Nitrite (Continued)	5. Check that the level of ammonium chloride-EDTA solution in the column is down to the top of the granules.	5a. If the level is too high, drain the excess into the beaker.	
	6. Pour the prepared nitrate working standard into the reduction column.	6a. Since the column will not hold the total amount, add the final amount after the first 15 ml has passed through the column.	
	7. Using the screw clamp (see Figure 1) adjust the collection rate to 7-10 ml per minute.	7a. The clamp should be slowly opened until a collection rate of 7-10 ml per minute is achieved. 7b. A collection rate of 7-10 ml of solution per minute should be carefully maintained throughout the collection process to assure complete reduction of nitrate in the sample.	
	8. Collect the reduced working standard until the level of solution is 0.5 cm above the top of the granules.		
	9. Close the screw clamp to stop the flow.		
	10. Discard the entire reduced working standard.	10a. The column is now activated.	
	11. Measure about 40 ml of ammonium chloride-EDTA solution.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Reduction of Nitrate to Nitrite (Continued)	12. Pour the 40 ml into the column.		
	13. Repeat steps 8 and 9.	13a. The nitrate standard should now be "washed off" the column.	
3. Reduction of Working Standards	1. Pipet 25.0 ml of the lowest concentration of nitrate working standard into a small Erlenmeyer flask.	1a. A 250 ml flask is good for this purpose. 1b. Label the flask. 1c. Begin with the 0.00 mg/liter solution.	
	2. Add 75 ml of the dilute ammonium chloride-EDTA solution to the same flask.	2a. Use a 100 ml graduated cylinder.	
	3. Mix nitrate working standard thoroughly by swirling the contents of the flask.		
	4. Place a short graduated cylinder under the reduction column.	4a. You need to measure 25 ml of solution in the graduate.	
	5. Pour the prepared nitrate working standard into the reduction column.	5a. Since the column will not hold the total amount, add the final amount after the first 15 ml has passed through the column.	
	6. Using the screw clamp (see Figure 1) adjust the collection rate to 7-10 ml per minute.	6a. The clamp should be slowly opened until a collection rate of 7-10 ml per minute is achieved. 6b. A collection rate of 7-10 ml of solution per minute should be carefully maintained throughout the collection process to assure complete reduction of the nitrate in the nitrate working standard.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Reduction of Nitrate to Nitrite (Continued)	7. Discard the first 25 ml of solution which is collected.	7a. This discard portion serves to "wash off" solution remaining in the column from any previous pass-through.	
	8. Replace the graduate with the rinsed, air-dried flask used for this standard.		
	9. Collect the remaining portion of the reduced standard in the original flask.	9a. Close the screw clamp when the level of solution is about 0.5 cm above the granules. 9b. About 70 ml should be in the flask.	
	10. Analyze the reduced standard immediately after collection from the reduction column.	10a. While one solution is passing through the column you should proceed to color development of the previous solution that has already been reduced. Color development (Section G) must begin within 15 minutes after reduction.	
	11. Repeat steps 1 through 10 for each of the prepared working nitrate standards.	11a. Proceed from the least concentrated to the most concentrated standard. 11b. Label each receiver flask.	
G. Color Development of Reduced Nitrate Working Standards	1. Use a 50.0 ml pipet to remove a 50.0 ml aliquot from flask #1 (0.00 mg/liter NO_3-N).	1a. By using a propipet the aliquot can remain in the pipet during the next two steps. 1b. Aliquots of each of the working standards should have been passed through the reduction column as described in the previous section (Section F). 1c. The reduced working standards should be analyzed as soon as possible after the reduction and in no case should they be allowed to stand for more than 15 minutes after reduction before color development is begun.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Color Development of Reduced Nitrate Working Standards (Continued)	2. Discard the remainder of the nitrate reduced working standard.		
	3. Shake flask dry.	3a. Do not rinse the flask.	
	4. Add the 50.0 ml working standard back to same flask from which it was removed.	4a. If you find the technique in steps 1-4 too difficult, transfer the 50.0 ml to a different flask.	
	5. Add 2.0 ml of the color reagent to the 50.0 ml of working standard.	5a. Use a 2.0 ml volumetric pipet.	
	6. Mix thoroughly by swirling.		
	7. Allow the working standard to stand until color develops.	7a. The reduced working standard should be allowed to stand for at least 10 minutes but not more than two hours before doing Procedure L, Spectrophotometric Measurements.	
	8. Repeat steps 1 through 7 for each of the reduced working standards.	8a. Start with least concentrated solution and proceed to most concentrated. 8b. Rinse the 50.0 ml pipet thoroughly after each standard.	
H. Analysis of Samples for Nitrate Reduced to Nitrite			
1. Dilution of Samples (if necessary)	1. Pipet 25.0 ml of unknown sample into 50 ml volumetric flask.	1a. Potable water samples will usually require no dilution, while sewage samples may require dilution.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Analysis of Samples for Nitrate Reduced to Nitrite (Continued)	2. Dilute to volume with distilled water.	2a. If you need to dilute a sample, you must apply a dilution factor to the concentration found from a standard curve.	VII.H.1.2a (p. 44)
2. Adjustment of pH	1. Use a pH meter to adjust the pH of each sample to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.	1a. Put the 50 ml of sample in a small beaker so the pH electrode(s) is covered with solution. 1b. Make sure that pH meter is calibrated within this range. 1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter. 1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)	
3. Reduction of Nitrate to Nitrite in Samples	1. Aliquots of each of the samples should be passed through the reduction column as described in Procedure F.3, "Reduction of Working Standards."		
4. Color Development in Samples	1. Follow the steps in Procedure G, "Color Development."		
I. Preparation of Nitrite Working Standards			
1. Nitrite Working Standards	1. Prepare nitrite working standards by respectively pipetting the following volumes of nitrite standard solution into each of six 100 ml volumetric flasks.	1a. Label flasks. 1b. Use appropriate volumetric pipets (0.5 ml, 1.0 ml, 2.0 ml, 5.0 ml, 10.0 ml). 1c. The 0.00 solution which contains no nitrite (or nitrate) serves as the reagent blank for the nitrite standards and samples that are not passed through the column.	

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>I. Preparation of Nitrite Working Standards (Continued)</td><td>
<table>
<tr><th>To Flask No.</th><th>Add This Volume of Nitrite Standard Solution</th><th>For This Concentration of NO_2-N in mg/l</th></tr>
<tr><td>1</td><td>0.0 ml</td><td>0.00</td></tr>
<tr><td>2</td><td>0.5 ml</td><td>0.05</td></tr>
<tr><td>3</td><td>1.0 ml</td><td>0.10</td></tr>
<tr><td>4</td><td>2.0 ml</td><td>0.20</td></tr>
<tr><td>5</td><td>5.0 ml</td><td>0.50</td></tr>
<tr><td>6</td><td>10.0 ml</td><td>1.00</td></tr>
</table>
2. Dilute each of the flasks to volume with distilled water.
3. Use the working standards immediately after their preparation.</td><td></td><td></td></tr>
<tr><td>2. Adjustment of pH</td><td>1. Use a pH meter to adjust the pH of each of the working standards to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.</td><td>1a. Use a beaker small enough for this volume of standard to cover the pH electrode(s).
1b. Make sure that pH meter is calibrated within this range.
1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter.
1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Color Development of Nitrite Working Standards	1. Pipet 25.0 ml of each of the nitrite working standards into each of six clean 250 ml Erlenmeyer flasks.	1a. Use a 25.0 ml volumetric pipet. 1b. Label each flask. 1c. The nitrite working standards are not passed through the reduction column.	
	2. Add 75 ml of dilute ammonium chloride-EDTA solution to each of the nitrite working standards.	2a. Use a 100 ml graduated cylinder.	
	3. Mix each thoroughly by swirling each flask.		
	4. Use a 50.0 ml pipet to remove a 50.0 ml aliquot from flask #1 (0.00 mg/liter NO_2-N).	4a. By using a propipet the aliquot can remain in the pipet during the next two steps.	
	5. Discard the remainder of the standard from the flask.		
	6. Shake the flask dry.	6a. Do not rinse the flask.	
	7. Add the 50.0 ml nitrite working standard back to the same flask from which it was removed.		
	8. Add 2.0 ml of the color reagent to each nitrite working standard.	8a. Use a 2.0 ml volumetric pipet.	
	9. Mix thoroughly by swirling.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Color Development of Nitrite Working Standards (Continued)	10. Allow the working standards to stand until color develops.	10a. At least 10 minutes but no more than 2 hours should be allowed before doing Procedure L, Spectrophotometric Measurements.	
	11. Repeat steps 4 through 10 for each of the nitrite standards.	11a. Proceed from the least concentrated to the most concentrated standard. 11b. Rinse the 50.0 ml pipet thoroughly after each standard.	
K. Analysis of Non-reduced Samples for Nitrite			
1. Dilution of Samples (if necessary)	1. Pipet 25.0 ml of unknown sample into 50 ml volumetric flask.	1a. NOTE: Potable water samples will usually require no dilution, while sewage samples may require dilution.	
	2. Dilute to volume with distilled water.	2a. If you need to dilute a sample, you must apply a dilution factor to get a final answer.	VII.K.1.2a (p. 44)
2. Adjustment of pH	1. Use a pH meter to adjust the pH of each sample to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.	1a. Put the 50 ml of sample in a small beaker so the pH electrode(s) is covered with solution. 1b. Make sure that pH meter is calibrated within this range. 1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter. 1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)	
3. Color Development	1. Pipet 25.0 ml of sample into a clean 250 ml Erlenmeyer flask.	1a. Use a 25.0 ml volumetric pipet. 1b. Label the flask. 1c. The sample is not passed through the reduction column.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
K. Analysis of Non-reduced Samples for Nitrite (Continued)	2. Add 75 ml of the dilute ammonium chloride-EDTA solution to the same flask.	2a. Use a 100 ml graduated cylinder.	
	3. Mix the sample thoroughly by swirling.		
	4. Use a 50.0 ml pipet to remove a 50.0 ml aliquot from flask.	4a. By using a propipet the aliquot can remain in the pipet during the next two steps.	
	5. Discard the remainder of the solution from the flask.		
	6. Shake flask dry.	6a. Do not rinse the flask.	
	7. Add the 50.0 ml of sample back to same flask from which it was removed.		
	8. Add 2.0 ml of the color reagent to the same flask.	8a. Use a 2.0 ml volumetric pipet.	
	9. Mix the sample thoroughly by swirling.		
	10. Allow the sample to stand until color develops.	10a. At least 10 minutes but no more than 2 hours should be allowed before doing Procedure L, Spectrophotometric Measurements.	
	11. Repeat steps 1 through 10 for each sample.	11a. Rinse the 50.0 ml pipet thoroughly after each sample.	

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
L. Spectrophotometric Measurements			
1. Adjusting the Instrument	1. Consult the manufacturer's instructions for calibrating your particular instrument.	1a. Instrument must be warmed up for at least 10 minutes. 1b. There is an EMP on "Use of a Spectrophotometer."	
	2. Adjust the wavelength to 540 nm.		
	3. Check to make sure that the instrument reads infinite absorbance with no sample cell in the instrument.	3a. If it does not read infinite absorbance with no sample cell in it, adjust the instrument so that it does read infinite absorbance (see manufacturer's instructions). 3b. Use on and off switch to calibrate infinite absorbance.	
2. Reduced Nitrate Standards and Sample(s)	1. Use the reduced nitrate reagent blank to adjust the instrument to zero absorbance.	1a. Use 0.00 nitrate working standard reagent blank which has been passed through the column. 1b. Adjust to zero absorbance using the calibration knob.	
	2. Measure and record the absorbance of each reduced nitrate working standard.	2a. Use the nitrate working standards which have been passed through the column. 2b. Use data sheet provided.	IX.L.2.2b (p. 47)
	3. Measure and record the absorbance for each reduced sample.	3a. Use data sheet provided.	
3. Non-reduced Nitrite Standards and Sample(s)	1. Use the nitrite reagent blank (non-reduced) to adjust the instrument to zero absorbance.	1a. Use 0.00 nitrite working standard reagent blank. 1b. Adjust to zero absorbance using the calibration knob.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
L. Spectrophotometric Measurements (Continued)	2. Measure and record the absorbance of each non-reduced nitrite working standard.	2a. Use data sheet provided.	IX.L.3.2a (p. 47)
	3. Measure and record the absorbance for each non-reduced sample.	3a. Use data sheet provided.	
M. Preparation of Calibration Curve	1. Obtain an 8 1/2 x 11 inch piece of graph paper.		
	2. Label the longer side as the concentration axis.	2a. See Training Guide for an example of labeling the axis on a calibration curve.	VII.M.2a (p. 45)
	3. Label the shorter side as the absorbance axis.		
	4. Use the absorbance value and its corresponding nitrate concentration for each of the nitrate working standards to make a plot of absorbance versus concentration.	4a. Use the absorbances and concentrations recorded on the data sheet in Column B, "Total NO_2+NO_3-N." 4b. This will be the standard curve for reduced samples.	IX.M.4a (p. 47)
	5. On another piece of graph paper follow steps 1, 2, 3, and 4 using absorbance values and the corresponding nitrite concentrations for each of the nitrite working standards.	5a. Use the absorbances and concentrations recorded on the data sheet in Column D, "NO_2-N." 5b. This will be the standard curve for non-reduced samples.	IX.M.5a (p. 47)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
N. Checking Column Efficiency	1. Divide the absorbance value for the 1.00 mg/liter NITRATE (NO_3) working standard by the absorbance for the 1.00 mg/liter NITRITE (NO_2) working standard to obtain the column efficiency as follows:	1a. The abbreviation, abs is used to stand for absorbance.	
	$\frac{\text{abs of 1.00 mg/liter } NO_3 \text{ std}}{\text{abs of 1.00 mg/liter } NO_2 \text{ std}} \times 100 = \%\text{ efficiency}$		
	2. Divide the absorbance values for each of the other NITRATE (NO_3) working standards by the absorbance value for the corresponding NITRITE (NO_2) working standard to obtain a column efficiency value in each case as was done in the previous step.		
	3. Calculate the average value for the column efficiency.	3a. The average value for the column efficiency should be between 96% and 104%. If the average % efficiency does not fall in this range, another cadmium reduction column should be prepared and tested until the average column efficiency does fall in this range. 3b. For regeneration of a column, see Training Guide.	VII.N.3b (p. 43)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
O. Determination of mg/liter Nitrite Nitrogen Plus Nitrate Nitrogen in a Sample	1. Use the absorbance for the reduced sample and the standard curve for reduced samples ("Total NO_2+NO_3-N") to obtain the mg/liter of nitrite-N plus nitrate-N in the sample and record it in Column (A) on the data sheet provided.	1a. If the sample was not diluted (25 ml of sample is used), the mg/liter result is read directly from the nitrate standard curve. 1b. If the concentration of nitrate in the sample is too high for analysis, the sample must be diluted. The procedure is described in H.1 and involves diluting the sample to a 50 ml volume. In this case, the mg/liter result from the nitrate standard curve must be multiplied by a dilution factor which would be: $\text{Dilution Factor} = \frac{50\ \text{ml}}{\text{ml sample used in dilution}}$ 1c. The reduction process converts the nitrate-N initially present in the sample to nitrite nitrogen and the species analyzed is nitrite nitrogen. 1d. Any nitrite nitrogen initially present in the sample remains as nitrite nitrogen after the reduction. Thus the total nitrite analyzed is the sum of the nitrite initially present and the nitrite which has been formed by reduction of nitrate.	IX.O.1a (p. 47) VII.O.1b (p. 44)
P. Determination of mg/liter Nitrite Nitrogen in a Sample	1. Use the absorbance for the non-reduced sample and the standard curve for non-reduced samples ("NO_2-N") to obtain the mg/liter of nitrite-N in the sample and record it in Column (C) on the data sheet provided.	1a. If the sample was not diluted (25 ml of sample is used), the mg/liter result is read directly from the nitrite standard curve. 1b. If the sample was diluted to a 50 ml volume (as given in H.1), the mg/liter result read from the nitrite standard curve must be multiplied by a dilution factor which would be: $\text{Dilution Factor} = \frac{50\ \text{ml}}{\text{ml sample used in dilution}}$	IX.P.1a (p. 47) VII.P.1b (p. 44)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
Q. Calculation of mg/liter Nitrate Nitrogen in a Sample	1. Subtract the mg/liter of nitrite-N in the sample from the mg/liter of nitrite-N plus nitrate-N in the sample to obtain the concentration of nitrate-N.	1a. Since the procedure measures the total nitrite concentration in a sample, the nitrite concentration of samples must be determined before reduction and after reduction. The nitrate concentration of a sample is then determined by: NO_3-N = (NO_2+NO_3-N) TOTAL AFTER RE-DUC-TION - (NO_2-N) BEFORE REDUCTION These concentrations were recorded on the data sheet in Columns (A) and (C) respectively.	IX.Q.1a (p. 47)
	2. Record the answer in Column (E) on the data sheet provided.		
R. Calculation of mg/liter Nitrate in Sample	1. Multiply the value found for nitrate-nitrogen (NO_3-N) by a factor of 4.43. 2. Record the answer in Column (F) on the data sheet provided.	1a. (NO_3-N) x (4.43) = mg/liter Nitrate in sample. 1b. NO_3-N value was calculated in Procedure Q and recorded in Column (E).	IX.R.1b (p. 47)
S. Calculation of mg/liter Nitrite in Samples	1. Multiply the value found for nitrite-nitrogen (NO_2-N) by a factor of 3.29. 2. Record the answer in Column (G) on the data sheet provided.	1a. (NO_2-N) x (3.29) = mg/liter Nitrite in sample. 1b. NO_2-N value is found by using the calibration curve for non-reduced samples as in Procedure P and recorded in Column (C).	IX.S.1b (p. 47)

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V	Field and Laboratory Equipment
VI*	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII*	Safety
IX*	Records and Reports

Training guide materials are presented here under the headings marked. These standardized headings are used throughout this series of procedures.

INTRODUCTION

Section I

TRAINING GUIDE NOTE

The cadmium reduction procedure for nitrate-nitrite nitrogen provides a sensitive method for the determination of nitrate singly, or nitrite and nitrate combined in drinking, surface, and saline waters. The method is commonly used to determine both nitrate-N and nitrite-N in water samples.

The procedure described in this EMP is applicable for range of 0.01 to 1.0 mg/liter of nitrate-nitrite nitrogen. However, the range may be extended by appropriate sample dilution.

The test described in this instruction can be found in the 1974 EPA Methods Manual on page 201, entitled Nitrogen, Nitrate-Nitrite (Cadmium Reduction Method). Another reference which contains an acceptable procedure for this test is on page 423 of the 14th edition of Standard Methods.

The major sources of nitrogen entering the environment are: through the heavy application of nitrogenous fertilizers which cause agricultural runoffs, as the end products of aerobic stabilization of organic nitrogen, in domestic sewage, through animal and plant processing wastes, in animal manure, through the atmosphere and in various types of industrial effluents.

While nitrogen is essential to our survival (as in the make-up of amino acids and proteins), when it exists as nitrate and nitrite it can be toxic. A limit of 10 mg/l nitrate-N and 1 mg/l nitrite-N is recommended for public water sources. The desirable criteria is virtually 0 mg/liter.

In ruminant animals (i.e. cows) nitrates may be internally reduced by bacteria present in the rumen to nitrites. The nitrites have been found to be toxic to these animals. Dr. Joptha E. Campbell, (Chief, Food Chemistry Unit, Milk and Food Research, Environmental Sanitation Program, Public Health Service, U.S. Department of H.E.W., Cincinnati, Ohio, 1968) has reported methemoglobinemia in cattle receiving water containing 2.790 mg/liter of nitrate.

Nitrates in high concentrations have also been found to stimulate vegetative growth under favorable conditions. Heavy undesirable growth in fresh water can lead to eutrification of important waterways.

REFERENCES/RESOURCES

1. Methods for Chemical Analysis of Water and Wastes, 1974, EPA-MDQARL, Cincinnati, Ohio 45268, p. 201.
2. Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, New York, p. 423.
3. Federal Water Pollution Control Administration Water Quality Criteria, U.S. Government Printing Office, Washington, D.C. 1968.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.	Samples should be analyzed for nitrate nitrogen as soon as possible after sampling to avoid any change in nitrogen balance due to biological activity. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. Samples should be preserved with sulfuric acid if they are to be held more than 24 hours. To preserve samples for analysis, add 2.0 ml of concentrated sulfuric acid per liter of sample and store at 4°C.	

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

C.2.19c
N.3b

Check the column efficiency when it is suspected that column efficiency is decreasing, as indicated by suspected low concentration levels. Prepare working standard nitrate solutions, and pass them through the column. (Begin at E. Preparation of Nitrate Working Standards.) If the absorbance for the known concentration does not give an average between 96% and 104% of your standard curve value for reduced nitrate standards of equivalent concentration, the column must be reactivated.

REACTIVATION OF COLUMN

1. Empty cadmium granules from column into a clean beaker.
2. Wash with distilled water 3 times.
3. Add enough dilute HCl to cover granules.
4. Swirl contents.
5. Decant HCl.
6. Wash with distilled water 3 times.
7. Add 100 ml $CuSO_4$ solution to granules.
8. Swirl contents of beaker for approximately 5 minutes until the blue color fades to colorless.
9. Decant liquid leaving the granules.
10. Repeat steps 7, 8, and 9 until a very fine brown-red precipitate forms.
11. Wash granules with distilled water (approximately 10 times) until precipitate is removed.
12. Place granules on the 60 mesn sieve.
13. Shake to remove the small particles (the particles which remain on the sieve are the ones you want.)
14. Repack column (packing must be loose).
15. Standard curve using nitrate working standards must be re-established.
16. Check column efficiency as described in N, Checking Column Efficiency.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
H.1.2a K.1.2a O.1b P.1b	Since a dilution is only part sample, when the absorbance reading obtained for it is converted to a concentration using a calibration curve, the concentration obtained is only that of the dilution. To obtain the mg/liter concentration of the sample, the mg/liter concentration of the dilution must be multiplied times the amount of dilution (must be multiplied times the dilution factor). For a 1/2 dilution (25 ml sample/50 ml total volume) the dilution factor would be 2 (the dilution is only half sample). For a 1/5 dilution (10 ml of sample/50 ml total volume) the dilution factor would be 5. Below is a table of some dilution factors when the sample is diluted to a 50 ml volume.	

ml of Sample per 50 ml Total Volume	Amount of Dilution	Dilution Factor
25	1/2	2
10	1/5	5
5	1/10	10
1	1/50	50
0.5	1/100	100
0.05	1/1000	1000

The dilution factor for any dilution may be calculated by dividing the ml of sample used in the dilution into 50:

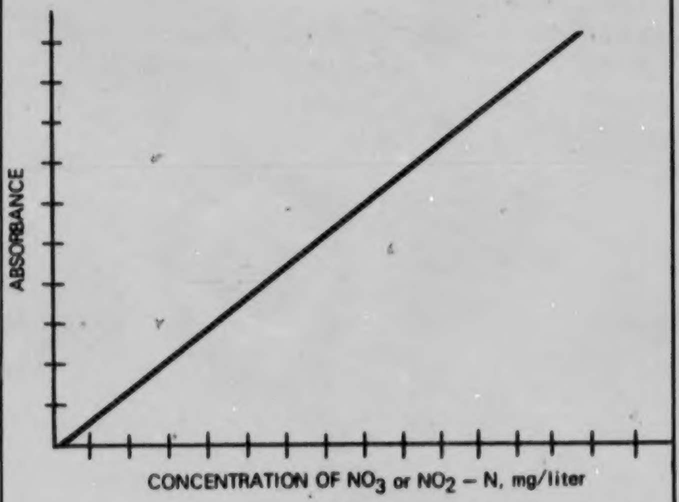
$$\text{Dilution Factor} = \frac{50\text{ ml}}{\text{ml sample used in dilution}}$$

Ex. 2 ml of sample diluted to 50 ml

$$\frac{50}{2} = 25$$

The dilution factor for this dilution would be 25.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
M.2a	A calibration curve is prepared by plotting the measured absorbance of each of the working standard versus the concentration in the working standard as shown below.	



	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.2.1d	Cadmium metal is highly toxic thus caution must be exercised in the use of cadmium. Cadmium metal should never be handled directly since cadmium has been shown to have cumulative effects. Rubber gloves should be used whenever cadmium must be handled. A mask should be worn during the filing of cadmium and the filing should be done in a hood. The waste cadmium should be disposed of in an appropriate manner which conforms to Federal, State and local pollution control regulations.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	You will need the following Key to use the Example Data Sheet found on the next page:	
	KEY TO DATA SHEET	
L.2.2b M.4a	(B) Record the absorbances of the column-reduced nitrate working standards and of the column-reduced sample(s) in Column (B).	
L.3.2a M.5a	(D) Record the absorbances of the non-reduced nitrite working standards and of the non-reduced sample(s) in Column (D).	
O.1a	(A) Read the mg/liter (concentration) of Total NO_2+NO_3-N in the column-reduced sample(s) from the corresponding calibration curve and record the answer(s) in Column (A).	
P.1a	(C) Read the mg/liter (concentration) of NO_2-N in the non-reduced sample(s) from the corresponding calibration curve and record the answer(s) in Column (C).	
Q.1a	(E) Subtract: Value (A) - Value (C) = Value (E)	
R.1b	(F) Multiply: Value (E) x 4.43 = Value (F)	
S.1b	(G) Multiply: Value (C) x 3.29 = Value (G)	

EXAMPLE DATA SHEET

See Key on Page No. 7-47

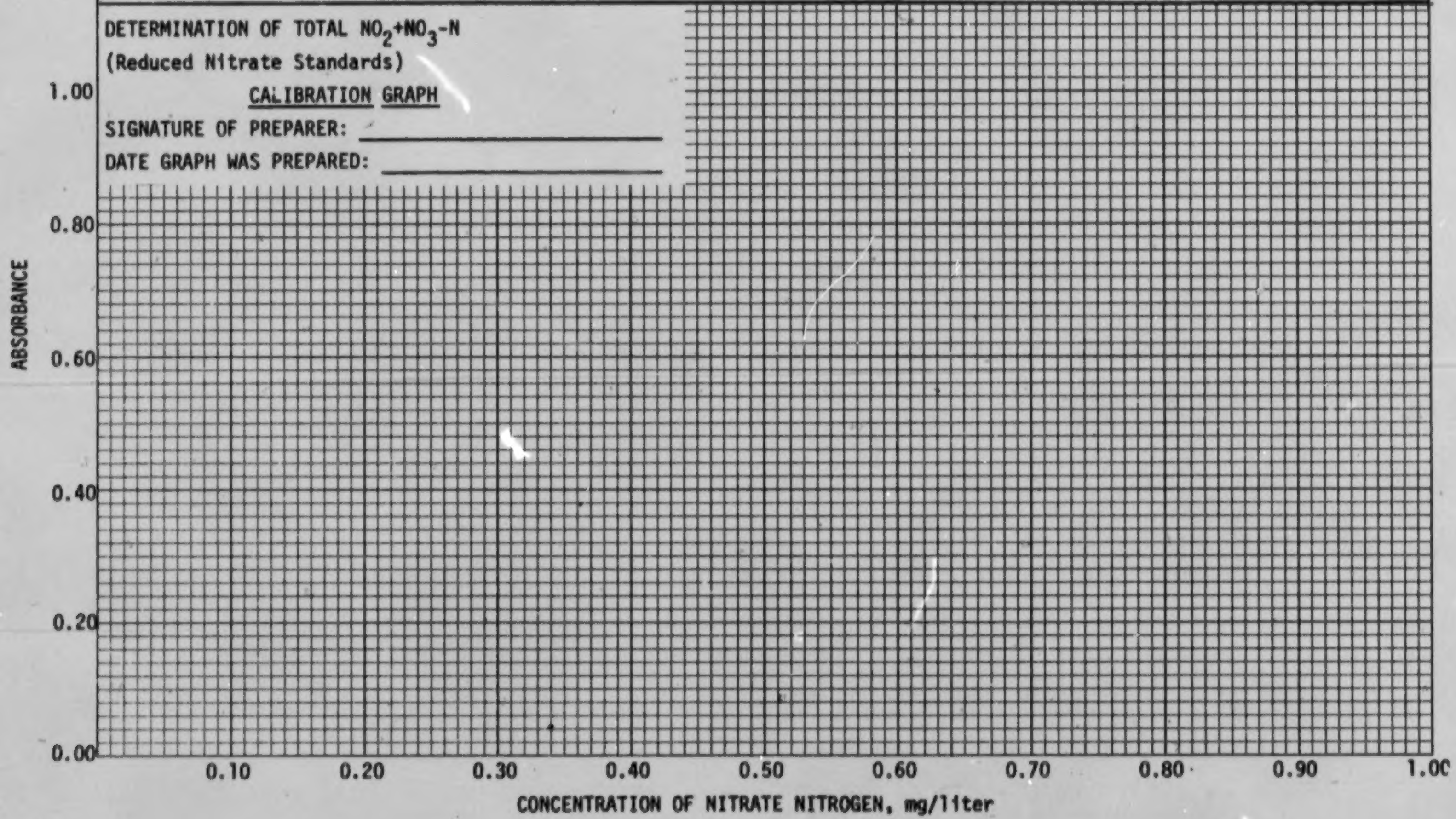
SAMPLE NUMBER	mg/liter TOTAL NO_2+NO_3-N (A)	ABSORBANCE OF TOTAL NO_2+NO_3-N (B)	mg/liter NO_2-N (C)	ABSORBANCE NO_2-N (D)	mg/liter NO_3-N (E)	mg/liter NO_3 (F)	mg/liter NO_2 (G)
Reduced Nitrate Working Standards							
2	0.05				0.05	0.22	
3	0.10				0.10	0.44	
4	0.20				0.20	0.89	
5	0.50				0.50	2.22	
6	1.00				1.00	4.43	
Reduced Sample(s)							
Non-reduced Nitrite Working Standards							
2			0.05				0.16
3			0.10				0.33
4			0.20				0.66
5			0.50				1.65
6			1.00				3.29
Non-reduced Sample(s)							

DETERMINATION OF TOTAL NO_2+NO_3-N
(Reduced Nitrate Standards)

CALIBRATION GRAPH

SIGNATURE OF PREPARER: ______________________

DATE GRAPH WAS PREPARED: ______________________

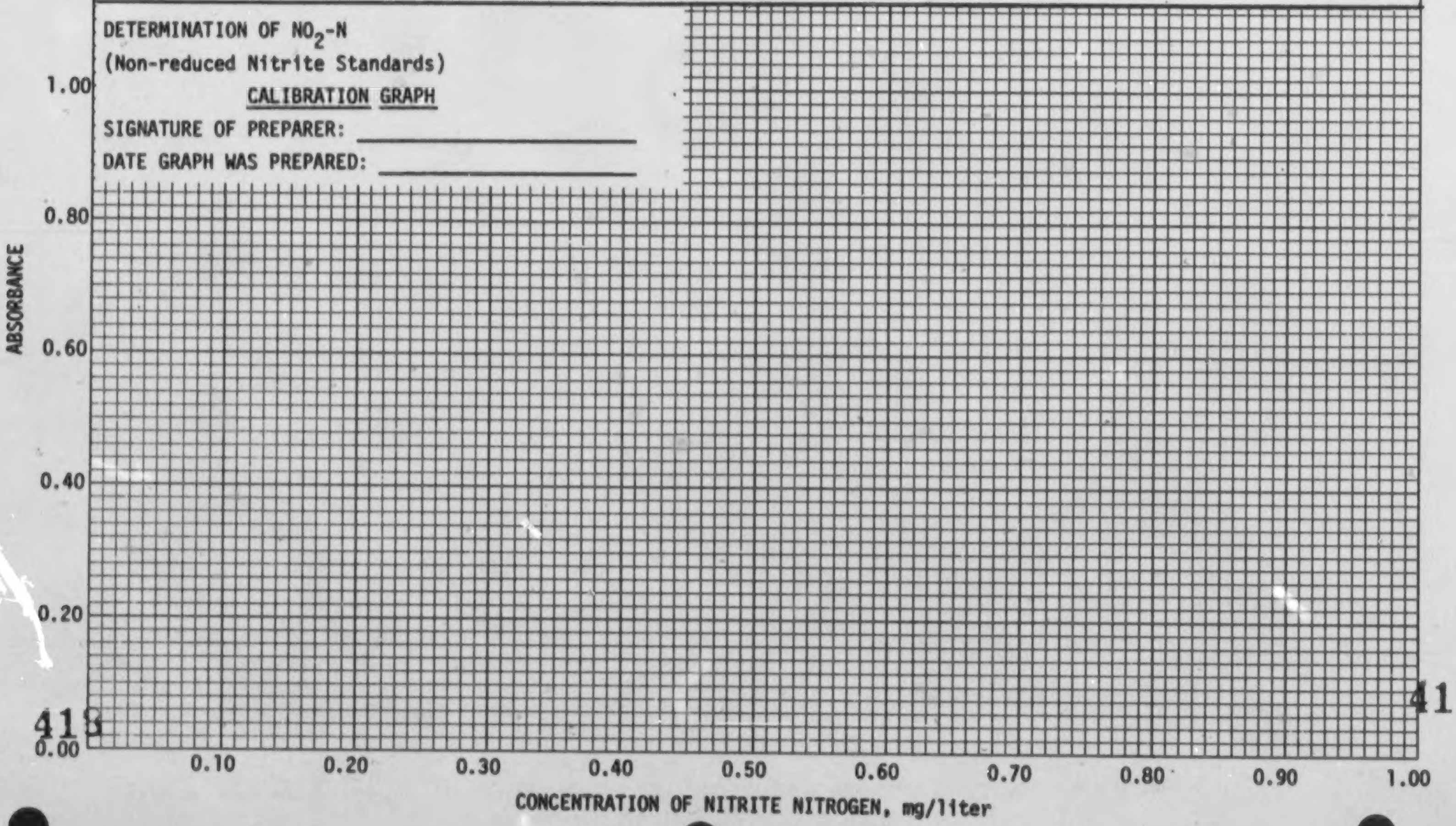


DETERMINATION OF NO_2-N
(Non-reduced Nitrite Standards)

CALIBRATION GRAPH

SIGNATURE OF PREPARER: ______________________

DATE GRAPH WAS PREPARED: ______________________



A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF OIL AND GREASE

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.og.EMP.1b.12.75

EFFLUENT MONITORING PROCEDURE: Determination of Oil and Grease

This operational procedure was developed by:

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EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

M.S. - Chemistry

1-1/2 years Industrial Chemist

4 years additional Graduate School

4 years college Chemistry Instructor

1-1/2 years DHEW - Air Pollution Program, Chemist

4-1/2 years DI - EPA, Chemist-Instructor

EFFLUENT MONITORING PROCEDURE: Determination of Oil and Grease

1. Analysis Objectives:

The operator will be able to perform an oil and grease determination on a sewage sample.

2. Brief Description of Analysis:

The sample is shaken in a separatory funnel with 1,1,2-trichloro-1,2,2-trifluoroethane, $C_2F_3Cl_3$, Freon TF or Genolsov D. The solvent and water do not dissolve in each other, and after the shaking, they separate and form two layers, with the solvent on the bottom. During the shaking, the oil and grease are taken from the water layer into the solvent layer, because the oil and grease are more soluble in the solvent than in water. The solvent is transferred to a previously weighed distilling flask. This process of shaking the water with solvent, the taking of the oil and grease into the solvent, and the separation of the solvent, is called extraction. The extraction is repeated two more times. All three solvent portions are combined in the distilling flask and evaporated. The flask is again weighed. The increase in weight is due to the oil and grease in the sample.

The method cannot distinguish between oil and grease, because both are soluble in the solvent, The two components are treated as one. Other solvent soluble materials may also be present and contribute to a result higher than it should be.

3. Applicability of this Procedure:

a. Range of Concentration:

5 to 1000 mg/liter extractable material

b. Pretreatment of Samples:

The Federal Register Guidelines do not specify any pretreatment.

c. Treatment of Interferences in Samples:

The Source of Procedure* does not note any interferences to this determination.

*Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, Ohio, p. 229

EFFLUENT MONITORING PROCEDURE: Determination of Oil and Grease

General Description of Equipment Used in the Process

A. Capital

1. Analytical balance (200 g capacity)
2. Still, or other source of distilled water
3. Source of vacuum (water aspirator or vacuum pump)
4. Hot water bath (80°C temperature needed)
5. Oven (103°C temperature needed)
6. Refrigerator, 4°C (for storing samples which will not immediately be analyzed after collection)
7. Hot plate (must have continuous setting between its lower and upper limit; cannot have only low, medium and high settings)
8. Steam bath (large enough to accommodate at least 1 distilling flask, 125 ml size)

B. Reusable

1. Brushes (for cleaning glassware)
2. Brush (for cleaning balance)
3. Laboratory apron
4. Safety glasses
5. Pen or pencil
6. Notebook (for recording data)
7. Centigrade thermometer (for taking readings at 70°C and 80°C)
8. Distilling flask, 125 ml, with a 24/40 ground glass neck (Corning number 4100 is an example) One flask is used for each determination.
9. Glass stoppered bottle, 1 liter
10. Grease pencil (for marking bottle)
11. Desiccator (large enough to hold at least one 125 ml distilling flask)
12. Crucible tongs (may be used in place of lintless tissues)
13. Graduated cylinders, 10 ml and 50 ml
14. Erlenmeyer flask, 125 ml
15. Glass stoppered bottle, 50 ml capacity
16. Ring stand
17. Funnel, 60°, 100-150 mm
18. Ring (to support the funnel)
19. Separatory funnel with Teflon stopcock, 2 liter
20. Ring (to support the separatory funnel)
21. Clamp (to fit neck of distilling flask)
22. Rubber stopper and glass tubing for preparing suction device; see figure 2
23. Beakers, 1000 ml (1), 100-150 ml (2)
24. Glass Stoppered bottle (for storing cleaning solution if prepared)
25. Beaker, 250 ml (for preparing cleaning solution)

B. Reusable (Cont'd.)

26. Rubber stopper to fit the distilling flask, item 8 above; see figure 2.
27. Fifteen inches of pyrex glass tubing (6 mm size); see figure 2
28. Gas and laboratory burner to bend the glass tubing; see figure 2
29. File to cut the glass tubing; see figure 2

C. Consumable

1. Concentrated sulfuric acid, H_2SO_4, or concentrated hydrochloric acid, HCl. (Either acid may be used in the determination. Concentrated sulfuric acid, H_2SO_4, may be needed for cleaning glassware.)
2. Sodium dichromate, $Na_2Cr_2O_7$ (for cleaning glassware)
3. Detergent (for cleaning glassware)
4. 1,1,2-trichloro-1,2,2-trifluoroethane*
5. Desiccant (enough to cover the bottom of the desiccator)
6. Lintless tissues (may be used in place of crucible tongs)
7. Whatman number 40 filter paper (to fit the funnel in B.17)
8. pH sensitive paper (for measurement at pH 2)
9. Anhydrous sodium sulfate, Na_2SO_4
10. Matches

*Freon 113 is a general name used by E. I. DuPont de Nemours, Inc., for the above solvent. TF and PCA are two specific grades of Freon 113. TF is the better of the two. Genosolv D is the name used by Allied Chemical Company for the above solvent. Either Freon TF or Genosolv D may be used in the determination.

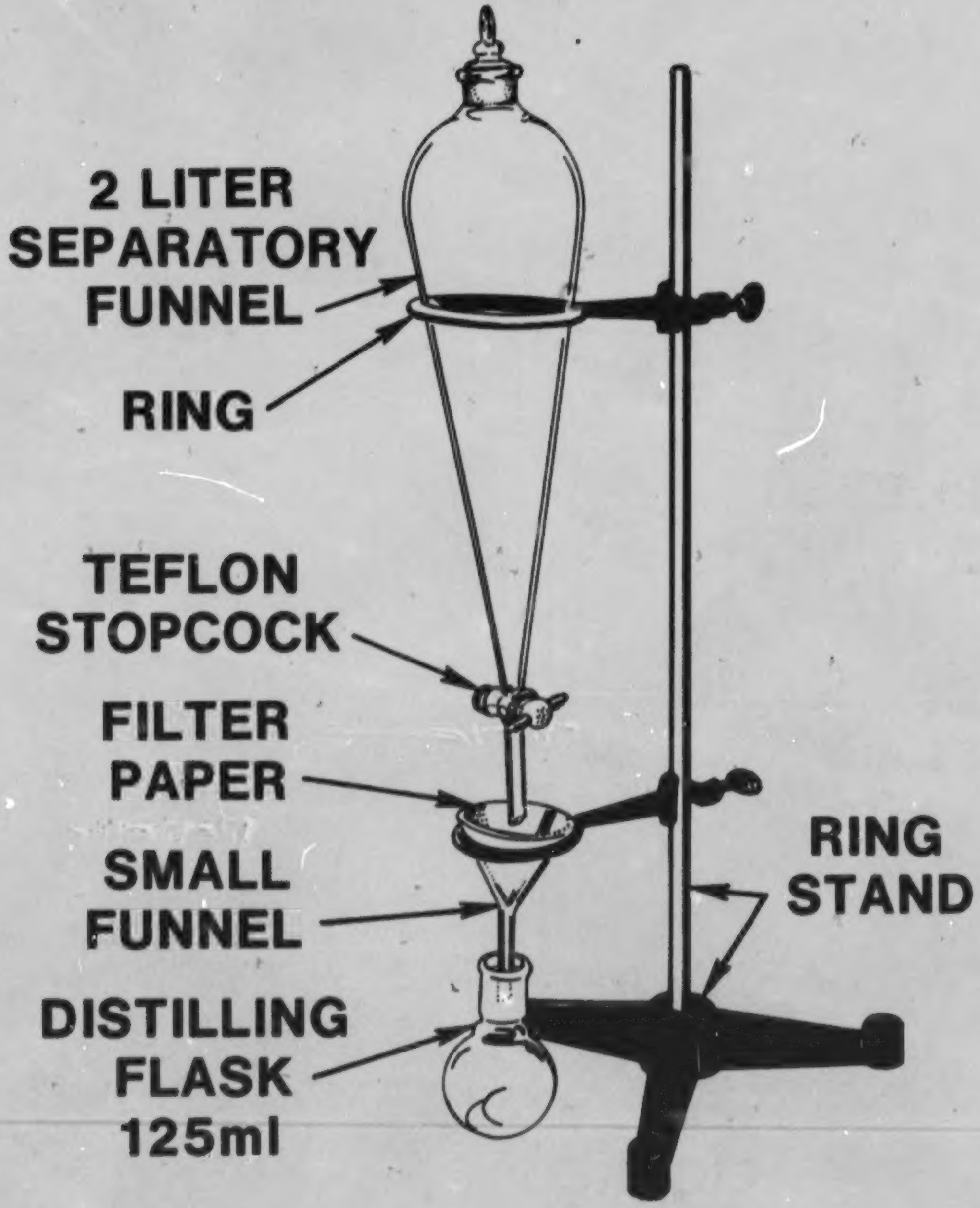
2 LITER SEPARATORY FUNNEL
RING
TEFLON STOPCOCK
FILTER PAPER
SMALL FUNNEL
DISTILLING FLASK 125ml
RING STAND

FIGURE 1

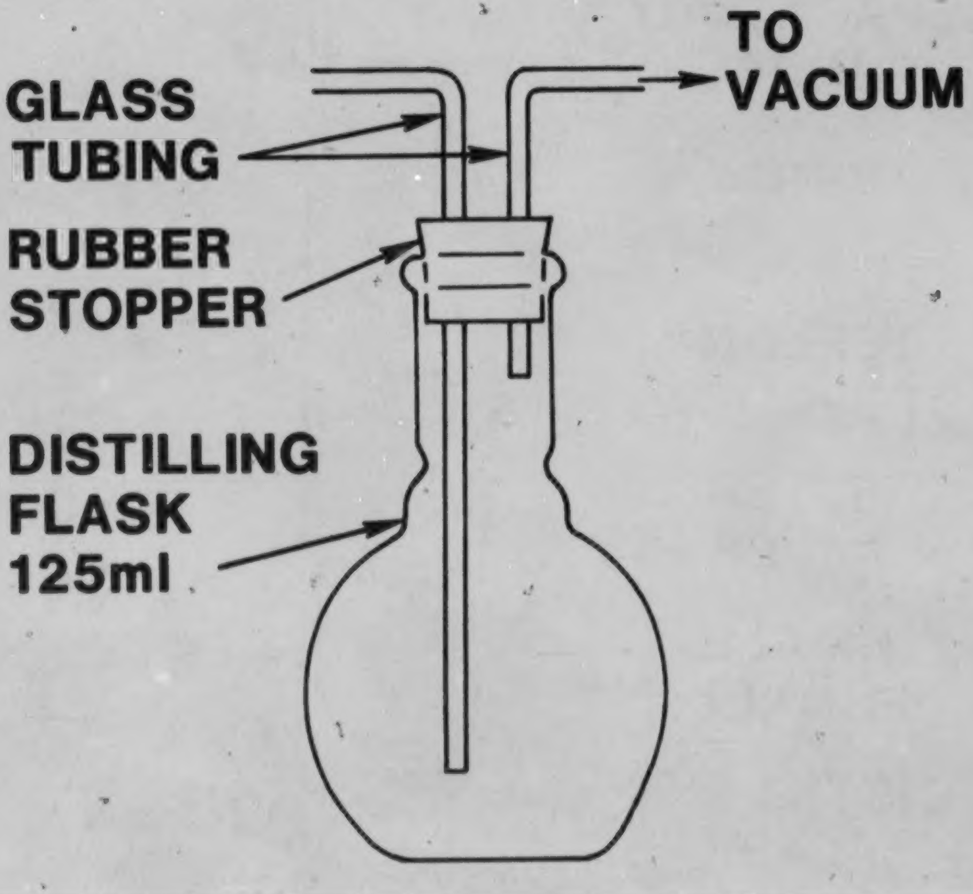
TO VACUUM
GLASS TUBING
RUBBER STOPPER
DISTILLING FLASK 125ml

FIGURE 2

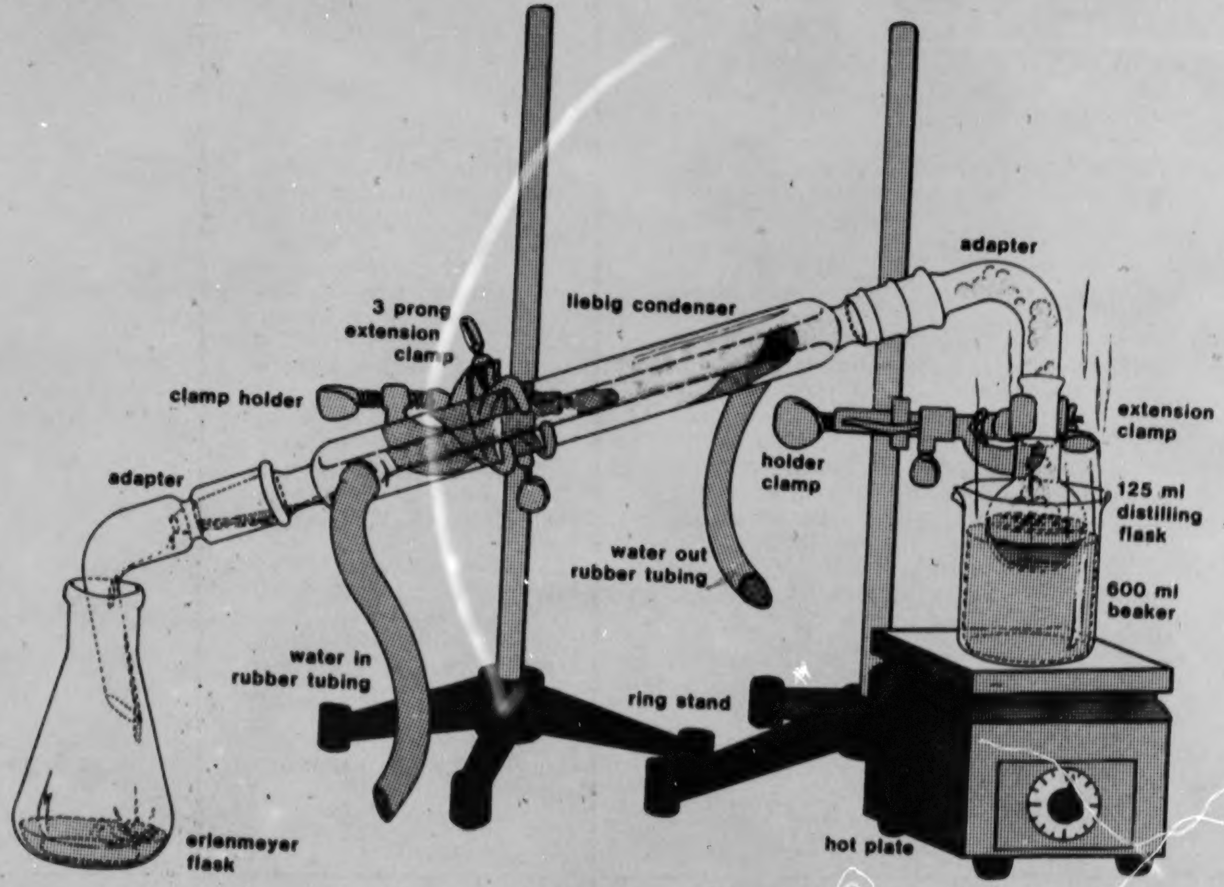
adapter
3 prong
extension
clamp
liebig condenser
clamp holder
extension
clamp
holder
clamp
adapter
125 ml
distilling
flask
water out
rubber tubing
600 ml
beaker
water in
rubber tubing
ring stand
erlenmeyer
flask
hot plate

FIGURE 3

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			I (p. 24)
1. Cleaning of glassware	1. Clean the 125 ml distilling flask and all other glassware.		V.A.1.1 (p. 25)
	2. Rinse all of the glassware with distilled water.		
	3. Allow all of the glassware to drain dry.		
	4. Rinse the 125 ml distilling flask with 20 ml of 1,1,2-trichloro-1,2,2-trifluoroethane (Freon TF or Genosolv D.	4a. For the remainder of this procedure the symbol TF/D will be used to mean this particular solvent. 4b. Use a graduated cylinder to measure the TF/D.	V.A.1.4 (p. 26)
	5. Allow the flask to drain dry.	5a. Proceed with preparation of the sample container and desiccator while waiting.	
2. Sample container	1. Pour 1 liter of distilled water into a 1 liter glass stoppered bottle.	1a. Measure the water with a graduated cylinder, 500 ml or 1000 ml size.	
	2. Place a grease pencil mark on the outside of the bottle at the 1 liter level	2a. There must be at least 1 inch space between the bottom of the glass stopper and the surface of the water. 2b. So that no oil will be lost by clinging to the stopper. 2c. And to allow room for the addition of reagents later in the determination. 2d. If there is less than 1 inch space, a larger bottle (marked at the 1 liter level) must be used.	
	3. Empty the water.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation (continued)	4. Allow the bottle to drain thoroughly.		
	5. Rinse the glass stoppered bottle with about 20 ml of TF/D.	5a. This TF/D may be disposed of by pouring it into a small beaker and allowing it to evaporate in a well ventilated area.	
	6. Hold the bottle upside down.		
	7. Lean the bottle up against some part of the laboratory bench top.	7a. Make sure the bottle will not fall over. 7b. The bottle should not be standing vertically. 7c. The heavy TF/D vapors must be able to escape from the mouth of the bottle.	
3. Desiccator	1. Prepare a desiccator for use.	1a. It must be large enough to hold at least one 125 ml distilling flask. 1b. The desiccator size will depend on the number of flasks to be held. 1c. One flask is required for each sample and blank determination.	
4. Distilling flask	1. Wipe the clean dry 125 ml distilling flask thoroughly with lintless tissues.	1a. To remove all finger prints. 1b. From this step on, until the determination has been completed, always handle the flask with lintless tissues or crucible tongs.	
	2. Dry the flask in an oven.	2a. For 1 hour at 103°C.	
	3. Cool the flask in a desiccator.	3a. For 30 minutes. 3b. Store the flask in the desiccator until needed.	
5. Stopper and glass tubing suction fitting	1. Drill 2 holes in the rubber stopper.	1a. To accommodate the 6 mm glass tubing; see figure 2.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation (continued)	2. Cut, bend, and fire polish the glass tubing.	2a. See figure 2.	
	3. Insert it through the holes in the rubber stopper.	3a. See figure 2.	
B. Reagent Preparation			
1. Sulfuric acid, H_2SO_4, 50% by volume	1. Measure 10 ml of distilled water.	1a. Use a graduated cylinder.	
	2. Pour it into a 125 ml Erlenmeyer flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	3. Measure 10 ml of concentrated sulfuric acid, H_2SO_4.	3a. Use a graduated cylinder. 3b. Concentrated hydrochloric acid, HCl, may be substituted for the concentrated sulfuric acid, H_2SO_4.	
	4. Pour about 1/2 of the acid slowly down the inside of the Erlenmeyer flask.	4a. Caution: Heat will be generated.	
	5. Gently swirl the flask to mix.		
	6. Pour the rest of the acid into the flask.	6a. Caution: Heat will be generated.	
	7. Gently swirl the flask to mix.		
	8. Allow the mixture to cool to room temperature.		
	9. Store the 50% sulfuric acid, H_2SO_4, in a small glass stoppered bottle.	9a. Of about 50 ml capacity. 9b. Five ml are needed for each determination. 9c. Larger quantities of the 50% acid (hydrochloric may be substituted) may be prepared if needed.	
C. Sample			
1. Collection	1. Fill the glass stoppered bottle to the 1 liter mark with sample.	1a. Collect the sample directly in the bottle so as to minimize loss of oil/grease by the use of an intermediate container.	V.C.1.1 (p. 26)
	2. Measure 5 ml of 50% by volume sulfuric acid, H_2SO_4.	2a. Use a graduated cylinder. 2b. Fifty percent by volume hydrochloric acid, HCl, may be substituted.	
	3. Add the acid to the sample bottle.		
	4. Gently swirl the bottle to Mix the acid and sample.		

EFFLUENT MONITORING PROCEDURE: Determination of Oil and Grease

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample (continued)	5. Check the pH of the acidified sample.	5a. Use pH sensitive paper. 5b. The pH must be 2 or less.	
	6. If the pH is not 2 or less, add 5-10 more drops of the 50% acid.		
	7. Swirl the bottle and again check the pH as before.	7a. With the stopper off. 7b. Repeat the acid addition, mixing, and pH check until the pH is 2 or less.	
2. Preservation	1. If the analysis will not be done immediately, store the acidified sample in a refrigerator at 4°C.	1a. For no longer than 24 hours. Otherwise, the analytical result may be unreliable.	
D. Procedure			
1. Extraction	1. Mount a 2 liter separatory funnel on a ring stand.	1a. The separatory funnel should have a Teflon stopcock. 1b. Use a ring.	
	2. Tighten the screw or clamp which holds the stopcock in place.	2a. A loose stopcock can cause loss of the sample by leakage.	
	3. Close the stopcock.		
	4. Pour the acidified sample into the separatory funnel.	4a. Use a funnel of about 75 mm diameter.	
	5. Measure 30 ml of TF/D.	5a. Use a graduated cylinder.	
	6. Pour it into the sample bottle.		
	7. Swirl the sample bottle.	7a. To thoroughly rinse the inside of the bottle with the TF/D.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	8. Pour the TF/D from the sample bottle into the separatory funnel.	8a. Pour the TF/D carefully so that any solids present are transferred to the separatory funnel.	
	9. Stopper the funnel.		
	10. Holding one hand over the stopper, lift the funnel out of the ring stand.		
	11. Carefully turn the funnel upside down.	11a. The stopper is pointed down. Be sure the tip of the funnel is not pointed toward your face.	
	12. Slowly open the stopcock.	12a. A hissing sound may be heard.	
	13. Close the stopcock.		
	14. Shake the funnel gently for about 5 seconds.		
	15. Slowly open the stopcock.	15a. A hissing sound may be heard.	
	16. Close the stopcock.		
	17. Shake the flask gently for about 5 seconds.		
	18. Slowly open the stopcock.	18a. A hissing sound may be heard.	
	19. Close the stopcock.		
	20. Shake the funnel vigorously for 2 minutes.		
	21. Place the separatory funnel back in the ring stand.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	22. Remove the stopper.		
	23. Allow the TF/D and water layers to separate.	23a. The TF/D layer will be under the water layer. 23b. There may be some bubbles at the point where the water and TF/D layers meet. 23c. These bubbles should break after a few minutes standing. 23d. If the shaking was extremely vigorous, an emulsion may have formed; that is, the water and TF/D molecules are so well mixed that they will separate only after long standing.	
	24. While the layers are separating, weigh the 125 ml distilling flask.	24a. Which had been stored in the desiccator. 24b. See the example data sheet on page 27. 24c. Use an analytical balance to weigh the flask.	
	25. Mount a 60° funnel (about 50 mm size) under the tip of the separatory funnel.	25a. The tip of the separatory funnel should extend down about one-half inch into the separatory funnel.	
	26. Fold a piece of Whatman number 40 filter paper to fit into the small funnel.	26a. The size of the filter paper will depend on the size of the funnel.	
	27. Place it in the funnel.		
	28. Place a 100-150 ml beaker under the tip of the small funnel.		
441	29. Pour about 10 ml of TF/D into a second 100-150 ml beaker.		
	30. Slowly pour the TF/D into the small funnel.	30a. The entire surface of the filter paper must be wet. 30b. The TF/D will evaporate from the filter paper rapidly.	442

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	31. When all of the TF/D has drained through the small funnel, the TF/D may be disposed of.	31a. By evaporation in a well ventilated area.	
	32. Place the previously weighed distilling flask under the tip of the small funnel.	32a. The tip of the small funnel should extend down into the neck of the flask about 1 inch (see figure 1).	
	33. Examine the separatory funnel and note whether the TF/D and water layers have separated so to form a sharp line between the two layers.	33a. No clear answer can be given as to how long the layers may take to separate. As little as a few minutes may suffice. 33b. About one-half hour would be the longest practical time one should wait before deciding to use the anhydrous sodium sulfate, Na_2SO_4 (see step 34).	
	34. If they have not, pour about 1 g. of anhydrous sodium sulfate, Na_2SO_4, into the small funnel.	34a. Estimate the 1 g. 34b. Omit step 34 if the two layers have separated. 34c. If there is doubt as to whether or not the two layers have separated properly, use the anhydrous sodium sulfate, Na_2SO_4.	
	35. Open the stopcock on the separatory funnel slowly.	35a. The TF/D should flow slowly from the separatory funnel into the small funnel, through the sodium sulfate (if used), through the filter paper, and into the distilling flask.	
	36. When the water layer is about to enter the hole through the stopcock, close the stopcock.	36a. A drop or two of the TF/D should remain in the funnel with the sample. 36b. There may be some scum clinging to the inside walls of the separatory funnel. It should be left in the separatory funnel.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	37. Repeat steps 5 through 23.	37a. The sample is still in the separatory funnel.	
	38. Place the small funnel (the same one used before) and filter paper under the tip of the separatory funnel.	38a. If anhydrous sodium sulfate, Na_2SO_4, was used in the first filtration, it is not necessary to remove it from the small funnel, even if the TF/D and water layers have cleanly separated. 38b. If anhydrous sodium sulfate, Na_2SO_4 was not used in the first filtration, it may be necessary to use it now, if the TF/D and water layers have not cleanly separated.	
	39. Place the distilling flask under the tip of the small funnel.	39a. The tip of the small funnel should extend down into the distilling flask about 1 inch. 39b. The TF/D from the first extraction is still in the flask.	
	40. Repeat steps 35 and 36.		
	41. Repeat steps 5 through 23.	41a. The sample is still in the separatory funnel.	
	42. Repeat steps 38 and 39.	42a. The TF/D from the second extraction is also still in the flask.	
	43. Repeat steps 35 and 36.	43a. The distillation flask now contains the TF/D from all three extractions.	
	44. Pour about 10 ml of TF/D into a small beaker.	44a. The same one used in step 28 or 29.	
	45. Pour a few drops of the Freon on the tip of the separatory funnel.	45a. To rinse down any TF/D which may contain oil and grease.	
	46. Pour the rest of the TF/D slowly around the inside of the small funnel.	46a. The filter paper and sodium sulfate, if used, will be washed. 46b. The washings will pass into the distilling flask.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	47. The sample, sodium sulfate, Na_2SO_4, (if used), and filter paper may now be discarded.	47a. The sample remaining in the separatory funnel after extraction can be discarded now.	
2. TF/D removal	1. Fill a 1 liter beaker half full with tap water.	1a. The TF/D removal may be done in one of two ways. The method described in the remainder of this procedure involves evaporation, and therefore loss, of the TF/D. 1b. If the TF/D is distilled off, the TF/D may be recovered for reuse. The source of heat for the distillation should be a beaker of 70°C water on a hot plate. While the TF/D is distilling off, proceed with step 6 (see figure 3).	
	2. Place the beaker on a hot plate.	2a. In a hood or other extremely well ventilated area. 2b. A hood is preferable because of the danger of inhaling TF/D fumes.	
	3. Turn on the hot plate.		
	4. Adjust the hot plate so the temperature of the water is 70°C.	4a. Check the temperature with a thermometer. 4b. Because of air currents, it will probably not be possible to maintain the temperature at exactly 70°C.	
	5. Support the flask in the 70°C water.	5a. Use a clamp and ring stand. 5b. The lower third of the flask should be in the water. 5c. The TF/D will begin to boil and evaporate. 5d. If several determinations are being done at once, a larger water bath will be required.	
	6. While the TF/D is evaporating, set a steam bath at 80°C.	6a. It will take about 30 minutes for the TF/D to evaporate at 70°C. 6b. Use a thermometer to check the temperature.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	7. After the TF/D has evaporated at 70°C, place the flask in the 80°C steam bath.	7a. Because of air currents, it will probably not be possible to maintain a temperature of exactly 80°C. 7b. Only the lower third of the flask should be heated.	
	8. Heat the flask for 15 minutes.		
	9. Remove the flask from the steam bath.		
	10. Support the flask by means of a clamp and ring stand.		
	11. Attach the stopper with glass tubing (see figure 2).		
	12. Apply suction to the flask for 1 minute.	12a. While the flask is still warm.	
	13. Wipe the outside of the flask thoroughly with lintless tissues.	13a. To remove grease which may have been in the water of either of the two baths.	
	14. Place the flask in a desiccator to cool.	14a. For 30 minutes.	
E. Final Weighing	1. Remove the flask from the desiccator.		
	2. Weigh it.	2a. Use the same balance as before.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Blanks	1. Clean a 125 ml distilling flask.	1a. The same type as was used in the procedure. 1b. Steps 1 through 11 should be carried out in same manner as was used in the procedure.	
	2. Rinse it with TF/D.		
	3. Wipe it with lintless tissues.		
	4. Dry it at 103°C.	4a. For 30 minutes. 4b. Stand the flask upside down in the oven so the heavy TF/D vapors will escape.	
	5. Cool it in a desiccator.		
	6. Weigh it.	6a. Use an analytical balance.	
	7. Measure 100 ml of TF/D.	7a. Use a graduated cylinder.	
	8. Pour it into the distilling flask.		
	9. Evaporate the TF/D.	9a. Use the same technique as for the sample.	
	10. Cool the flask in the desiccator.		
	11. Weigh it.	11a. The initial and final weights should be within 0.0002 g of each other. (This difference was suggested by the EPA laboratory which wrote the 1974 EPA oil and grease method of analysis.) 11b. If the two weights are not within 0.2 mg of each other, check for faulty laboratory techniques.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Blanks (continued)	12. Calculate the value of the blank.	12a. Blank, D = E-F D = value of the blank in grams E = weight of the flask after evaporation of the 100 ml of TF/D (in grams) F = weight of the empty flask (in grams) 12b. Example calculation: Weight of the flask after evaporation of the 100 ml of TF/D (E) = 54.6961 g Weight of the empty flask (F) = 54.6959 g Blank, D = 54.6961 g-54.6959 g = 0.0002 g	
G. Calculations	1. Calculate the mg of oil and grease per liter of sample.	1a. mg of oil and grease per liter of sample = [(A-B)-D] x 1000 x 1000/C 1b. A = the weight of the distilling flask + the oil/grease residue (in grams) B = the weight of the empty distilling flask (in grams) 1000 = a conversion factor to change milliliters to liters 1000 = a conversion factor to change grams to milligrams C = milliliters of sample D = value of blank (in grams); see F.12 for the calculation 1c. Example calculation: Weight of flask and the oil/grease residual = 54.7803 g (A) Weight of empty flask = 54.6961 g (B) Volume of sample = 1000 ml (C) Value of blank = 0.0002 g (D) $\frac{[(54.7803-54.6961)-0.0002] \times 1000 \times 1000}{1000} = 84.0$	

EFFLUENT MONITORING PROCEDURES: Determination of Oil and Grease

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII	Field and Laboratory Analyses
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

INTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	The terms oil and grease are not clearly defined. The definition depends on the procedure used. For example, the solvent used to extract the grease and oil, and the presence of extractables which are neither grease nor oil, will affect the results. Hydrocarbons, esters, oils, fats, waxes and high molecular weight fatty acids feel greasy and are associated with grease problems in wastewater treatment plants. Gasoline, heavy fuel and lubricating oils and asphalts are included in the term oil.	
	Oil and grease interfere with wastewater treatment by coating particles of organic matter, thus inhibiting oxygen transfer and stabilization by micro-organisms.	
	They can coat equipment, reducing its efficiency, and can cause a safety hazard on walkways and ladders.	
	The test dexcribed in this instruction can be found in the 1974 EPA Methods Manual on page 229. Another reference which has an acceptable procedure for this test for NPDES purposes is 14th ed. Standard Methods on page 515.	Methods for Chemical Analysis of Water and Wastes, 1974, EPA, MDQARL, Cincinnati, Ohio 45268, p. 229. Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, NY, p. 515.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required.	
	1. Pour 35 ml of distilled water in a 250 ml beaker.	13th Standard Methods, p. 135, section 2.c.2
	2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dichromate, $Na_2Cr_2O_7$, to the water.	
	3. Swirl the beaker until the sodium dichromate has dissolved.	
	4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve.	
	5. Pour the solution into a 2 liter beaker.	
	6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker. Caution: Use eyeglasses and protective clothing.	
	7. Stir the mixture thoroughly.	
	8. Store it in a glass stoppered bottle.	
	9. The cleaning solution should be at a temperature of about 50°C when it is used.	
	10. It may therefore be necessary to warm the cleaning solution.	
	11. When using the warm cleaning solution, fill the piece of glassware with the solution.	
	12. Allow it to soak for 2-3 minutes (or longer).	
	13. Pour the cleaning solution back into the storage bottle.	
	14. Rinse the piece of glassware ten times with tap water.	
	15. The cleaning solution may be reused until it turns green.	
	16. It should then be discarded.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.4	Toward the end of this determination, TF/D will be evaporated from the distjlling flask, and therefore lost. The TF/D may, however, be distilled from the flask and recovered for later reuse. This is the reason for using a distilling flask. (See fig. 3)	
C.1.1	Depending on how the plant outfall is constructed, there will probably be several ways in which the sample can be collected in the bottle. Whichever method is chosen, make sure that it is done in the same manner each time.	

EFFLUENT MONITORING PROCEDURE: Determination of Oil and Grease

Blank Determination

Weight of distilling flask after evaporation of the 100 ml of TF/D = E = ____________ grams

Weight of the empty distilling flask used to determine the blank = F = ____________ grams

Value of blank, D = E-F

Sample Determination

Weight of distilling flask + the oil/grease residue = A = ____________ grams

Weight of empty distilling flask used for the sample = B = ____________ grams

Volume of sample = C = ____________ milliliters

$$\text{Milligrams of oil/grease residue per liter sample} = \frac{[(A-B)-D] \times 1000 \times 1000}{C}$$

OTHER APPROVED ANALYTICAL PROCEDURES

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF AMMONIA BY AN AMMONIA SELECTIVE ION ELECTRODE

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Determination of Ammonia by an Ammonia Selective Ion Electrode

This Operational Procedure was developed by:

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POSITION	Chemist-Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.S. Chemistry
3 years - Research Chemist
13 years - Training Instructor

EFFLUENT MONITORING PROCEDURE: Determination of Ammonia by an Ammonia Selective Ion Electrode

1. Objective:

To place an Orion** ammonia electrode and specific ion meter into operation to make a determination of the ammonia concentration in an effluent sample.

2. Brief Description of Analysis:

Following a manual distillation of the sample at a pH of 9.5 the ammonia concentration is determined using an ammonia selective electrode and a specific ion meter. The procedure includes electrode assembly, membrane installation, and calibration of the meter.

3. Applicability of this Procedure:

a. Range of Concentration:

0.03 to 1.0 mg NH_3-N/liter

Information is given so the same stepwise procedure can be used for NH_3-N concentrations up to 1400 mg/liter.

b. Pretreatment of Samples:

The Federal Register Guidelines specify manual distillation of the sample at pH 9.5 unless sufficient acceptable proof exists to show that non-distilled samples yield comparable data. The distillation procedure is not included in this write-up because the step-wise directions are in the EMP, "Nitrogen, Ammonia Determination."

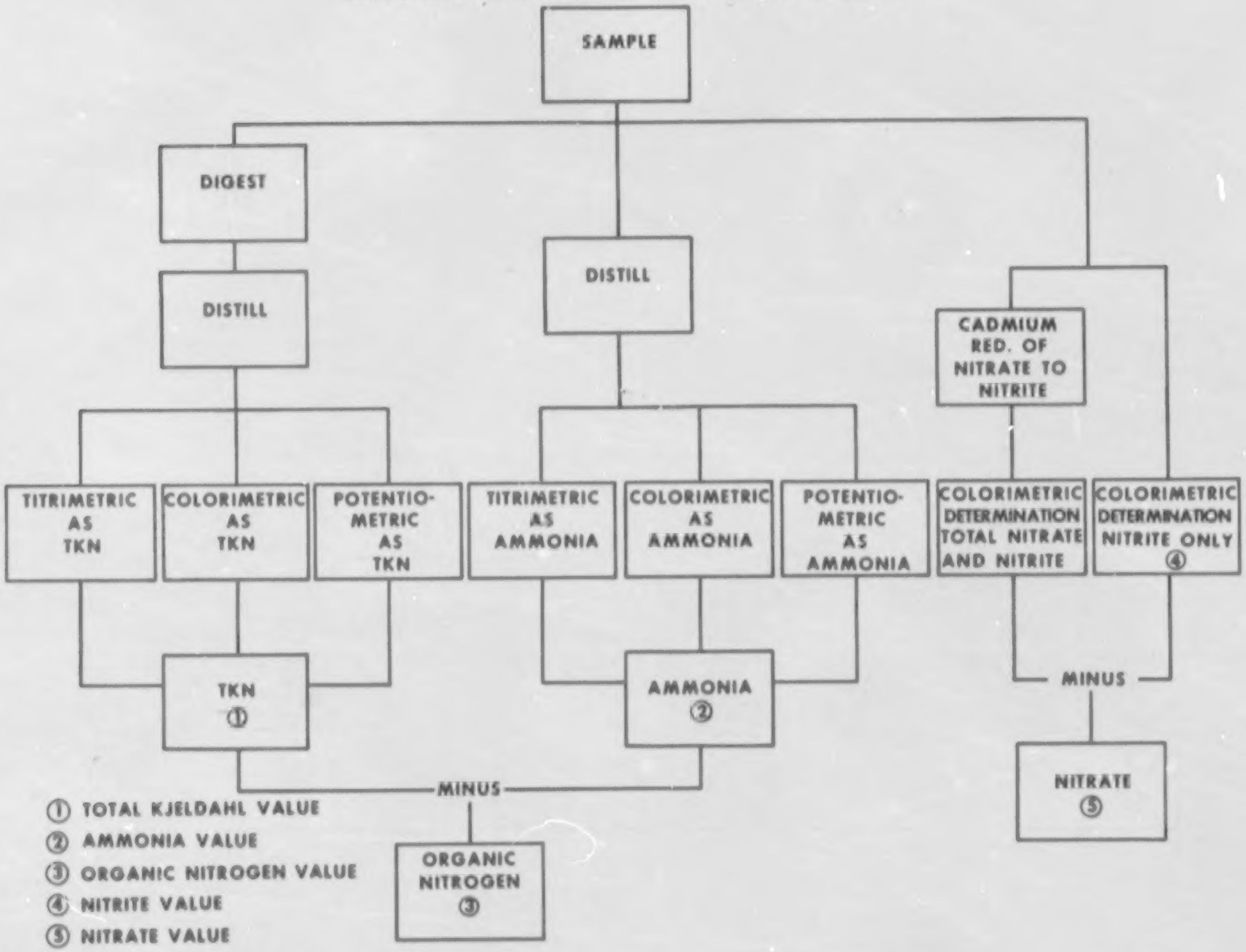
c. Treatment of Interferences in Samples:

Two interferences are listed in the Source of Procedure*. It notes that volatile amines in samples contribute to high results. However, no remedy is given so treatment for this interference is not included in this procedure. The other interference is the presence of mercury which forms a complex with ammonia to give low results. The Training Guide in this EMP includes remedies for this interference.

*Source of Procedure: Methods of Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, Ohio, p. 165
and
Instruction manual for Probe and Meter, Orion Research Inc., Cambridge, MA 02139.

**Mention of a particular brand name does not constitute endorsement by the U.S. Environmental Protection Agency

NITROGEN SERIES DETERMINATION FLOW SHEET


SAMPLE
DIGEST
DISTILL
DISTILL
CADMIUM RED. OF NITRATE TO NITRITE
TITRIMETRIC AS TKN
COLORIMETRIC AS TKN
POTENTIO-METRIC AS TKN
TITRIMETRIC AS AMMONIA
COLORIMETRIC AS AMMONIA
POTENTIO-METRIC AS AMMONIA
COLORIMETRIC DETERMINATION TOTAL NITRATE AND NITRITE
COLORIMETRIC DETERMINATION NITRITE ONLY ④
TKN ①
AMMONIA ②
MINUS
NITRATE ⑤
MINUS
ORGANIC NITROGEN ③

① TOTAL KJELDAHL VALUE
② AMMONIA VALUE
③ ORGANIC NITROGEN VALUE
④ NITRITE VALUE
⑤ NITRATE VALUE

EFFLUENT MONITORING PROCEDURE: Determination of Ammonia by an Ammonia Selective Ion Electrode

Equipment and Supply Requirements

A. Capital Equipment:

1. Orion Specific Ion Meter, Model 401, 407, or 407A
2. Orion Ammonia Electrode, Model 95-10
3. Magnetic stirrer
4. Analytical balance, 200 g capacity
5. Trip balance, 500 g capacity
6. Water still and ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin
7. Still for distillation of samples (For details see the EMP, "Determination of Total Kjeldahl Nitrogen," which contains this procedure.)

B. Reusable Supplies:

1. XXX beakers, 150 ml, two plus one for each sample
2. One cylinder, graduated, 100 ml
3. One flask, Erlenmeyer, graduated, 1000 ml
4. Three flasks, volumetric, 1000 ml
5. One flask, volumetric, 250 ml
6. One pipet, volumetric, 1 ml
7. Two pipets, volumetric, 10 ml
8. One pipet, volumetric, 25 ml
9. One pipet, volumetric, 100 ml
10. One pipet bulb
11. One plastic wash bottle
12. One pair safety glasses
13. One spatula, medium size
14. One laboratory apron

C. Consumable Supplies:

1. Sodium hydroxide, NaOH, reagent grade, 1 lb. unit
2. Ammonium chloride, NH_4Cl, analytical grade, 4 oz. unit
3. Brushes and soap to clean glassware
4. Wax marking pencil
5. Disposable paper wipers
6. Two plastic weighing boats

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
DETERMINATION OF AMMONIA			I (p. 23)
A. Sample Preservation			
1. Collection	1. Collect a minimum of 400 ml in a plastic or glass container.	1a. Because organic nitrogen is progressively formed by biological activity, the determination of ammonia is best made on a fresh sample.	
	2. Cool to 4°C.	2a. Sample may be held for 24 hours.	
2. Addition of preservative	1. If more storage time is needed, 2 ml of concentrated sulfuric acid, H_2SO_4, per liter may be added before cooling.	1a. When acid is added there exists the possibility of breakdown of organic nitrogen to form ammonia. This addition is done only if storage time in excess of 24 hours is expected.	
B. Equipment Preparation			
1. Glassware	1. Clean all glassware in suitable detergent.	1a. Distilled water should drain without leaving any droplets.	
	2. Rinse with ammonia-free distilled water.	2a. See section C.1.1a.	
2. Still cleaning	1. Clean the still until the distillate shows no trace of ammonia.	1a. For this procedure consult the Training Guide or the EMP, "Determination of Total Kjeldahl Nitrogen."	I.B.2 (p. 23) V.B.2.1a (p. 28)
3. Specific ion meter preliminary check	1. Check meter zero.	1a. With the instrument turned off the needle on the meter should point to the center of the scale. If not, a screw adjustment is located on the meter face.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Equipment Preparation (continued)	2. Turn function switch to the BATT position. See Figure 4.	2a. Figure 4 is in the Training Guide. 2b. The needle should swing past the green BATT OK area on the right side of the meter face. If the needle fails to pass the green area, replace the batteries.	V.B.3.2a (p. 26)
	3. Replace batteries if necessary.	3a. Replace with two 4.5 volt alkaline type batteries--NEDA #1306A (Mallory-#MN-1306, Everready-#523 or Burgess #AL 133). 3b. Place instrument face down and remove four recessed screws. Lift off rear panel and remove batteries. Check connections for corrosion and remove any if it exists. Replace batteries matching the marked polarity. Repeat battery test and if okay, replace panel.	
4. Specific ion meter operation check	1. Insert shorting strap in electrode connectors.	1a. The shorting strap is a single wire with the same type connectors that are on the electrode, one on each end. 1b. Insert large connector into large input jack on the instrument panel and small connector into small red input jack.	
	2. Turn function switch to any measuring position (not battery test).	2a. If the needle is not on scale, turn calibration control to bring the needle on scale. 2b. If after coming to rest in one position the needle does not remain stable, the instrument is not functioning properly and should be serviced.	
C. Reagent Preparation			
1. Distilled water	1. Prepare about six (6) liters of distilled water. This water should be free from ammonia.	1a. Pass distilled water through an ion-exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (continued)			
2. Ammonium chloride stock solution (1000 mg NH_3-N/liter)	1. Weigh out 3.819 g of ammonium chloride (NH_4Cl).	1a. Use an analytical balance.	
	2. Transfer the chemical to a 1 liter volumetric flask.		
	3. Add about 500 ml of water to the flask.	3a. Unless otherwise specified the term water means ammonia-free water.	
	4. Dilute to the volume mark with water.	4a. Label flask as ammonium chloride 1000 mg NH_3-N/liter. 4b. Mix well by shaking.	
3. Ammonium chloride intermediate solution (10 mg NH_3-N/liter)	1. Add about 500 ml of water to a 1 liter volumetric flask.		
	2. Pipet 10 ml of stock (1000 mg NH_3-N/liter) ammonium chloride solution into the flask.	2a. Use a 10 ml volumetric pipet.	
	3. Dilute to the volume mark with water.	3a. 1.0 ml = 0.01 mg NH_3-N. 3b. Label flask as ammonium chloride 10 mg NH_3-N/ liter. 3c. Mix well by shaking.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (continued)			
4. Ammonium chloride standard solution (1 mg NH_3-N/liter)	1. Add about 500 ml of water to a 1 liter volumetric flask.		
	2. Pipet 100 ml of the intermediate (10 mg NH_3-N/liter) ammonium chloride solution into the flask.	2a. Use a 100 ml volumetric pipet.	
	3. Dilute to the volume mark with water.	3a. Prepare dilution fresh daily. 3b. 1.0 ml = 0.001 mg NH_3-N. 3c. Label flask as ammonium chloride 1 mg NH_3-N/liter. 3d. Mix well by shaking.	
5. Ammonium chloride standard solution (0.1 mg NH_3-N/liter)	1. Add about 150 ml of water to a 250 ml volumetric flask.		
	2. Pipet 25 ml of the standard (1 mg NH_3-N/liter) ammonium chloride solution into the flask.	2a. Use a 25 ml volumetric pipet.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (continued)	3. Dilute to the volume mark with water.	3a. Prepare dilution fresh daily. 3b. 1.0 ml = 0.0001 mg NH_3-N. 3c. Label flask as ammonium chloride 0.1 mg NH_3-N/liter. 3d. Mix well by shaking.	
6. Sodium hydroxide solution, 10 M	1. Weigh out 400 g of sodium hydroxide (NaOH).	1a. CAUTION: This is a strong base and should be handled with care. Use safety glasses. 1b. Use a trip balance.	
	2. Dissolve the 400 g of sodium hydroxide in 800 ml of water in a 1 liter Erlenmeyer flask.	2a. CAUTION: A large amount of heat is liberated during dissolution.	
	3. Cool to room temperature.	3a. Allow cold tap water to run on the side of the flask.	
	4. Dilute to the 1000 ml volume line with water.	4a. This solution should be kept in a plastic container. 4b. Label container as sodium hydroxide, 10 M.	
D. Assembly of Electrode	1. Unscrew the top portion of the electrode through which the wire passes.	1a. See Figure 1 in the Training Guide.	V.D.1.1a (p. 24)
	2. Lift out top and attached inner body of electrode.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Assembly of Electrode (continued)	3. Place inner body on flat clean surface		
	4. Unscrew bottom portion of electrode outer body.		
	5. Remove O-ring, spacer, and old membrane.	5a. If this is the first use of the electrode, there will be no old membrane in place. The electrode is shipped dry and without a membrane. 5b. The O-ring is a red rubber ring. 5c. The spacer is a black plastic ring with a black O-ring recessed in a notch at one end around its inside diameter. The end which has the O-ring is placed toward the bottom of the bottom cap.	
	6. Remove a membrane from the the container with the tweezers.	6a. The membranes are packaged with a blue packing paper between each membrane. Discard the blue packing paper. 6b. The membrane should not be handled.	I.D.6a (p. 23)
	7. Place the new membrane in the bottom cap.	7a. With the "dimpled" side facing upward toward the inner body and the patterned side facing down toward the sample solution. See Figure 2 below.	

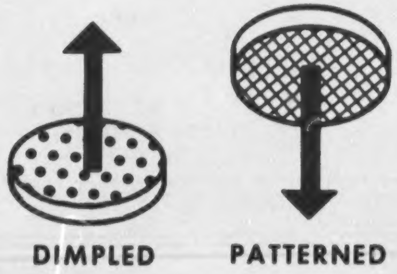


FIGURE 2

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Assembly of Electrode (continued)	8. Replace spacer.	8a. With its recessed O-ring down.	
	9. Replace O-ring.		
	10. Screw outer body into bottom cap.	10a. Do this by turning the outer body, not the bottom cap.	
	11. Fill outer body with filling solution provided by the manufacturer.	11a. It is best to put the filling spout on the bottle. This spout is provided with but not on the filling solution. 11b. Fill the outer body with filling solution to about 1 cm above the joint between the outer body and the bottom cap. If the outer body is overfilled, the excess will flow out of the vent hole when the inner body is replaced.	
	12. Screw top cap and inner body onto outer body.		
	13. Place assembled electrode into holder attached to rod on the meter.	13a. Electrode must be held at a 20° angle with respect to the vertical to prevent air bubble entrapment under the electrode. 13b. Orion Research Incorporated sells a holder (Cat. No. 920001A) which has the proper angle and will work with their Model 400 series Specific Ion Meters. See Figure 3 in Training Guide.	V.D.13b (p. 25)
	14. Plug the electrode cable into the meter.	14a. The electrode cable ends with an input jack and a pin jack. They should be connected to the input connector and reference electrode connectors respectively of the specific ion meter. 14b. The ammonia electrode does not require an external reference electrode. 14c. See Figure 4 in Training Guide.	V.D.14c (p. 26)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Assembly of Electrode (continued)	15. Lower electrode into about 100 ml of 0.1 M ammonia chloride solution.	15a. The solution can be put into a small beaker.	
	16. Allow electrode to stand for about one-half hour before use.		
E. Electrode Operation Check	1. Transfer 100 ml of 0.1 mg NH_3-N/liter standard solution to a 150 ml beaker.	1a. Use a 100 ml graduated cylinder. 1b. This is Reagent C.5.	
	2. Place the beaker on the stir plate and add the stir bar to the beaker.	2a. Samples and standards should be stirred using a magnetic stirrer. Some magnetic stirrers generate sufficient heat to change solution temperature. This effect can be minimized by placing a piece of insulating material on the stirrer (for example a piece of cork or a plastic petri dish). 2b. Samples and standards should be at the same temperature.	
	3. Lower the electrode into the standard solution.	3a. The solution should at least cover the joint between the bottom cap and the outer body. 3b. Make sure the stir bar does not hit the electrode.	
	4. Turn on stirrer.	4a. Provide a good mixing rate. However, do not stir solutions at so fast a rate as to cause a vortex to be formed.	
	5. Add 1 ml of the 10 M sodium hydroxide solution.	5a. The sodium hydroxide should be added at 1 ml of 10 M sodium hydroxide per 100 ml of (neutral pH 7) solution.	

(continued)

EFFLUENT MONITORING PROCEDURE: Determination of Ammonia by an Ammonia Selective Ion Electrode

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Electrode Operation Check (continued)		5b. The pH of any solution to be tested with the electrode must be above 11 after the addition of the sodium hydroxide. 5c. Caution: This is a caustic solution. Do not allow contact with skin. 5d. Use a 1 ml pipet. 5e. Do not add prior to electrode immersion.	
	6. Turn the specific ion meter function switch to MV EXP (Millivolts Expanded Scale).	6a. This is read from the meter on the blue scale on the #401; on the black scale on the #407 and 407A. The expanded mode has a ± 70 mv range. See Figure 5 in Training Guide.	V.E.6a (p. 27)
	7. After 30 seconds adjust the meter to the center scale.	7a. Turn the CALIB (calibration) knob and adjust the meter to obtain a reading of 0 (center scale) on the millivolt scale. See Figure 4 in Training Guide.	V.E.7a (p. 26)
	8. Turn the function switch to off.		
	9. Raise the electrode out of the sodium hydroxide solution.		
	10. Rinse the electrode with distilled water and blot dry with tissue.		
	11. Transfer 100 ml of 1 mg NH_3-N/liter standard solution to a 150 ml beaker.	11a. This is Reagent C.4.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Electrode Operation Check (continued)	12. Place the beaker on the stir plate and add stir bar to the beaker.	12a. The stir bar should be rinsed with distilled water between uses.	
	13. Lower the electrode into the standard solution.	13a. Make sure the stir bar does not hit the electrode.	
	14. Add 1 ml of the 10 M sodium hydroxide solution.	14a. Do not add prior to electrode immersion. 14b. Use same pipet as in the previous procedure.	
	15. Turn the function switch to MV EXP.		
	16. After 30 seconds read the meter.	16a. The reading should be taken from the same millivolt scale that was used to set the previous concentration. 16b. The reading should show a change of approximately 59 mv. This change (59 mv) will occur for every tenfold change in concentration because of the electrode make-up. 16c. If an mv reading near 59 mv is not obtained, check all standard dilutions and repeat all steps in section E. 16d. If continued failure to obtain an mv change near 59 mv occurs, contact the electrode manfacturer.	
	17. Turn function switch to off.	17a. Always set this position before lifting any electrode from the solution and when the meter is not actually measuring. This will extend the life of the batteries and protect the meter.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration			
1. Setting mid-scale range	1. Transfer 100 ml of the 0.1 mg NH_3-N/liter standard solution to a 150 ml beaker.	1a. The standards chosen should span the range that the sample concentration is expected to be in. 1b. With the standards prepared in this EMP a range will be covered from 0.01 to 1.0 mg NH_3-N/liter (0.01 to 1.0 ppm). 1c. Use a 100 ml graduated cylinder.	V.F.1.1a (p. 28)
	2. Place the beaker on the stir plate and add stir bar to the beaker.		
	3. Lower electrode into the standard solution.	3a. Make sure the stir bar does not hit the electrode.	
	4. Turn on stirrer.		
	5. Transfer 1.0 ml of the 10 M sodium hydroxide solution to the same beaker.	5a. Do not add prior to electrode immersion. 5b. Use a 10 ml graduated pipet.	
	6. Use appropriate range pH paper to check if the pH is greater than 11.	6a. If not,add more sodium hydroxide until pH is greater than 11.	
	7. Turn the instrument function switch to the monovalent anion position.	7a. This position is labeled differently on the various meters in the Orion 400 series. On the 401 and 407A it is marked x^-. On the 407 it is F^-. See Figure 4 in Training Guide.	V.F.1.7a (p. 26)
	8. After the meter stops drifting, read the meter.	8a. Response of the electrode will be faster for higher concentrations of ammonia and slower for lower concentrations. They can vary from less than 30 seconds to about 10 minutes.	

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration (continued)		8b. The reading should be taken from the concentration scale which is usually color coded to match the color of the monovalent position on the function switch. The scale is usually the topmost one and is logarithmically divided.	
	9. Use the calib (calibration) control and adjust the meter needle to read at center scale.	9a. The marking at center scale is different for the various meters. It is 100 for the 401 and 407 and 1 for the 407A. See Figure 5 in Training Guide. 9b. After the adjustment has been made, this center position will represent a concentration of 0.1 mg NH_3-N/liter.	V.F.1.9a (p. 27)
	10. Turn function switch to off.		
	11. Raise the electrode.		
	12. Rinse the electrode with distilled water and blot dry with tissue.		
2. Setting high-scale range	1. Transfer 100 ml of the 1.0 mg NH_3-N/liter standard solution to a 150 ml beaker.	1a. Use a 100 ml graduated cylinder.	
	2. Place the beaker on the stir plate and add stir bar to the beaker.		
	3. Lower electrode into the standard solution.	3a. Make sure the stir bar does not hit the electrode.	
	4. Turn on stirrer.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration (continued)	5. Transfer 1.0 ml of the 10 M sodium hydroxide solution to the same beaker.	5a. Do not add prior to immersion. 5b. Use a 10 ml graduated pipet.	
	6. Use appropriate range pH paper to check if the pH is greater than 11.	6a. If not, add more sodium hydroxide until pH is greater than 11.	
	7. Turn the function switch to the monovalent anion position.		
	8. After the meter stops drifting, read the meter.	8a. Use same uppermost scale as used for previous concentration.	
	9. Use the "Temp °C" control and adjust meter needle to read at far right position.	9a. For location of this control knob, see Figure 4 in Training Guide. 9b. Again the marking will vary by instrument. It will be 1000 on the 401 and 407 and will be 10 on the 407A. See Figure 5 in Training Guide. 9c. This position now represents a concentration of 1.0 mg NH_3-N/liter and the instrument has been adjusted to represent 0.01 mg to 1.0 mg NH_3-N/liter over the full scale of the meter face. 9d. Values below 0.03 mg NH_3-N/liter should be disregarded because of a deviation from normal response curve.	V.F.2.9a (p. 26) V.F.2.9b (p. 27)
	10. Turn function switch to off.		
	11. Recalibrate as needed.	11a. It is advisable to standardize electrodes 3 or 4 times a day by carrying out steps in section F.	

(continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration (continued)		11b. Always use fresh solutions of the standards for recalibration.	
G. Procedure	1. After the calibration has been completed, read each sample concentration by doing the following steps.	1a. Use 100 ml volumes in 150 ml beakers.	
	2. Rinse electrode.		
	3. Add 1.0 ml or more of sodium hydroxide solution after immersion of electrode until pH is greater than pH 11.		
	4. Read after drifting has stopped.	4a. Read concentration directly in mg NH_3-N/liter from the concentration scale. 4b. Do not adjust calib (calibration) or Temp °C (temperature compensator) controls after calibration. If they are changed, recalibrate instrument.	
H. Storage 1. Between readings	1. Immerse electrode in alkaline standardizing solution.	1a. You can use one of the standardizing solutions with 10 M sodium hydroxide which you used in F, calibration. 1b. The electrode should be immersed between measurements. 1c. Do not store in air.	V.H.1.1c (p. 28)

FFLUENT MONITORING PROCEDURE: Determination of Ammonia by an Ammonia Selective Ion Electrode

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Storage (continued)			
2. Overnight	1. Immerse electrode in ammonium chloride stock solution (1000 mg NH_3-N/liter).	1a. Without sodium hydroxide.	
3. Prolonged time	1. Disassemble electrode completely.		
	2. Rinse with distilled water.	2a. Rinse inner body, outer body and bottom cap.	
	3. Dry and reassemble.	3a. Without filling solution or membrane. 3b. Discard membrane.	
4. Membrane replacement	1. Follow steps under procedure D, Assembly of Electrode.		V.H.4 (p. 28)

EFFLUENT MONITORING PROCEDURE: Determination of Ammonia by an Ammonia Selective Ion Electrode

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	Ammonia has been of interest to both water and wastewater treatment plants for years. The content of ammonia in the effluent waters of a wastewater plant can indicate to the operator the efficiency of operation at which the plant is being run. Furthermore, ammonia can have a significant effect on the disinfection of water with chlorine. Consequently, monitoring the concentration of ammonia should be routine. The analysis of ammonia concentrations is also the basis for routine determinations of total nitrogen or of organic nitrogen in effluent samples.	
	The test described in this instruction can be found in the 1974 EPA Methods Manual on page 165. No other reference is cited in the Federal Register Guidelines. However, the referenced EPA Methods Manual in turn refers the analyst to the manufacturer's operating manual for the specific ion meter being used.	Methods for Chemical Analysis of Water and Wastes, 1974, EPA, MDQARL, Cincinnati, Ohio, 45268, p. 165.
B.2	Distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary. However, manual distillation will be required to resolve any controversies.	New Federal Register Guidelines, (1976), note #4.
	If the determination is to be run as part of the total Kjeldahl nitrogen determination, distillation must be carried out. Any mercury in the sample will form a complex with the ammonia and act as an interference. This will be taken care of in the distillation procedure by the addition of the sodium thiosulfate.	Methods for Chemical Analysis of Water and Wastes, 1974, EPA, MDQARL, Cincinnati, Ohio 45268, pg. 175.
	(If mercury is present and the sample is not to be distilled, add 0.2 g sodium thiosulfate to the sample to complex the mercury before the determination of ammonia).	op. cit. p. 160
D.6a	The ammonia electrode uses a membrane which will not allow water or ionic species to migrate across it. However, the ammonia dissolved in the sample can diffuse through the membrane until the concentration of the ammonia is the same on both sides of the membrane. The reference element, contained in the ammonia electrode itself, is the same as used in the chloride specific ion electrode. It senses the fixed level of the chloride in the ammonia chloride internal filling solution, thereby acting as a reference electrode for the ammonia electrode.	Instruction Manual for Ammonia Electrode Model 95-10, Orion Research, Inc., Cambridge, MA 02139

D.1.1a

ASSEMBLY INSTRUCTIONS

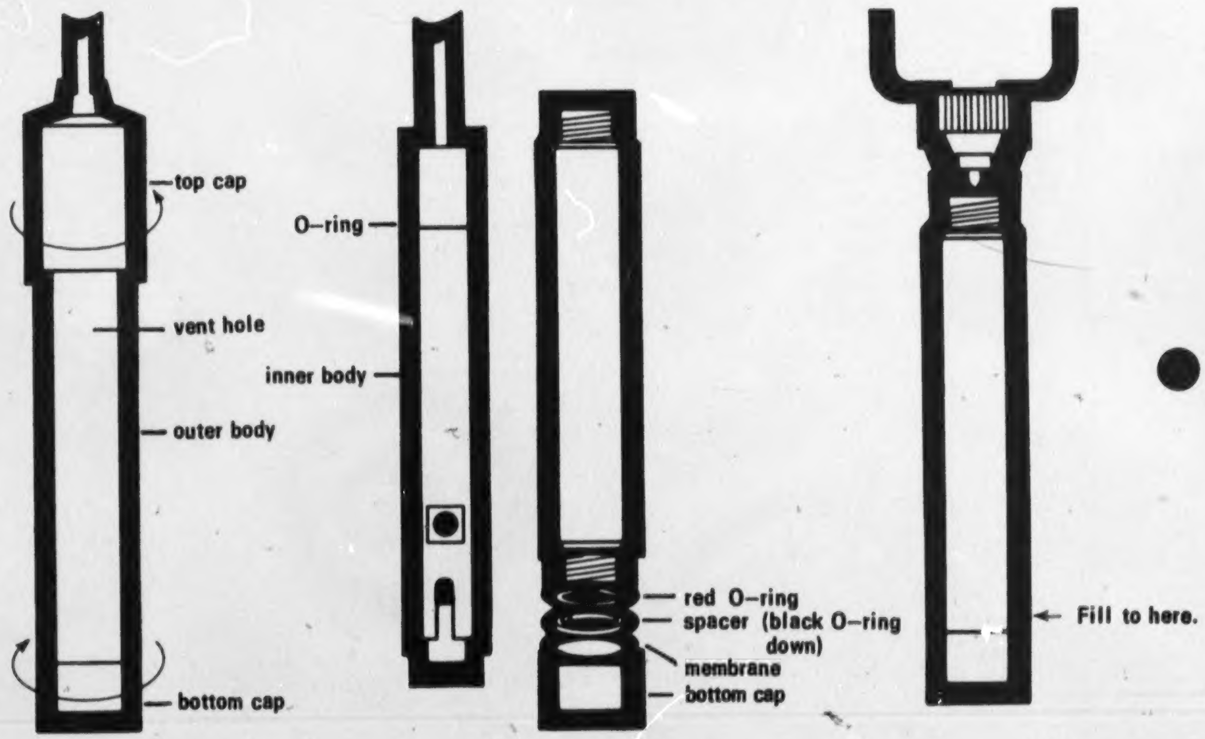

top cap
vent hole
outer body
bottom cap
O-ring
inner body
red O-ring
spacer (black O-ring down)
membrane
bottom cap
Fill to here.

FIGURE 1

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.13b		

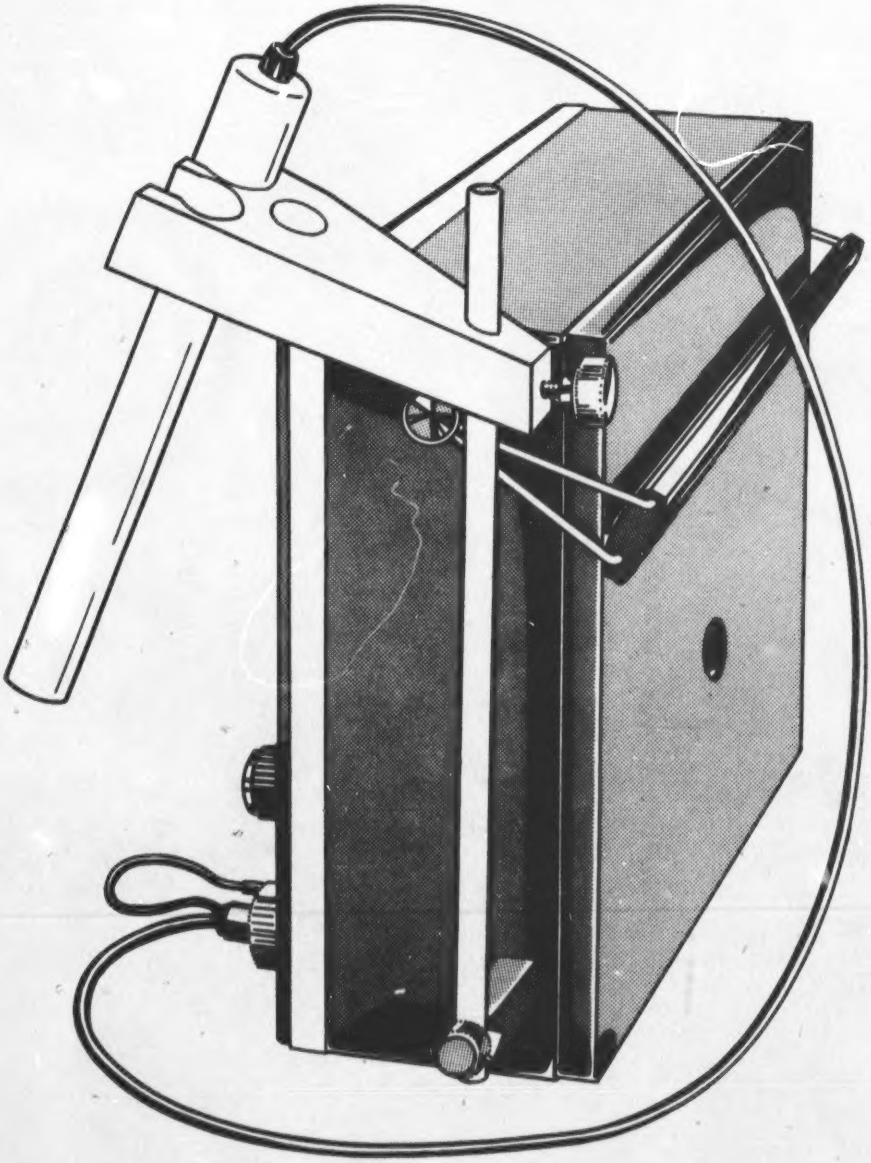


FIGURE 3

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.3.2a D.14c E.7a F.1.7a F.2.9a		

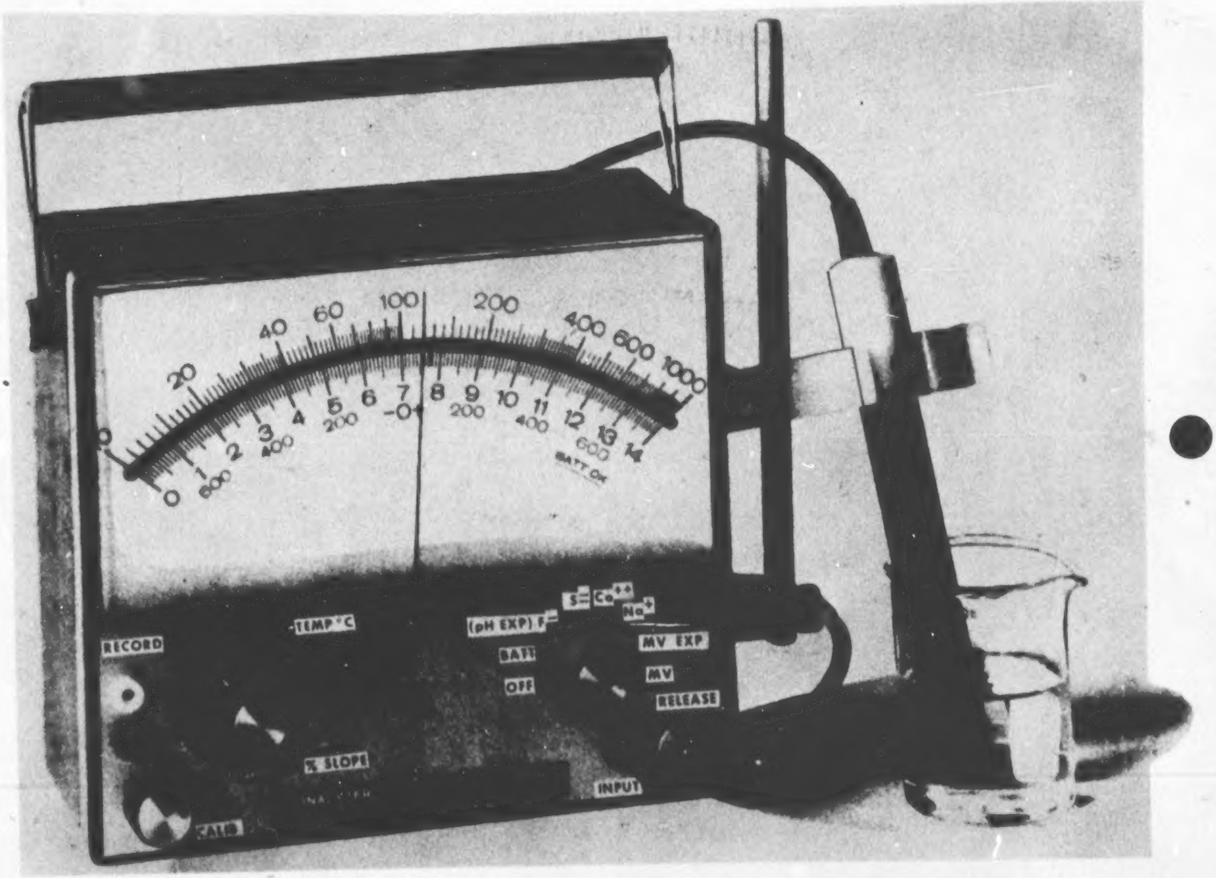

RECORD
TEMP °C
(pH EXP) F⁻
S⁼ Ca⁺⁺ Na⁺
BATT
OFF
MV EXP
MV
RELEASE
% SLOPE
CALIB
INPUT
BATT OK

FIGURE 4

E.6a
F.1.9a
F.2.9b

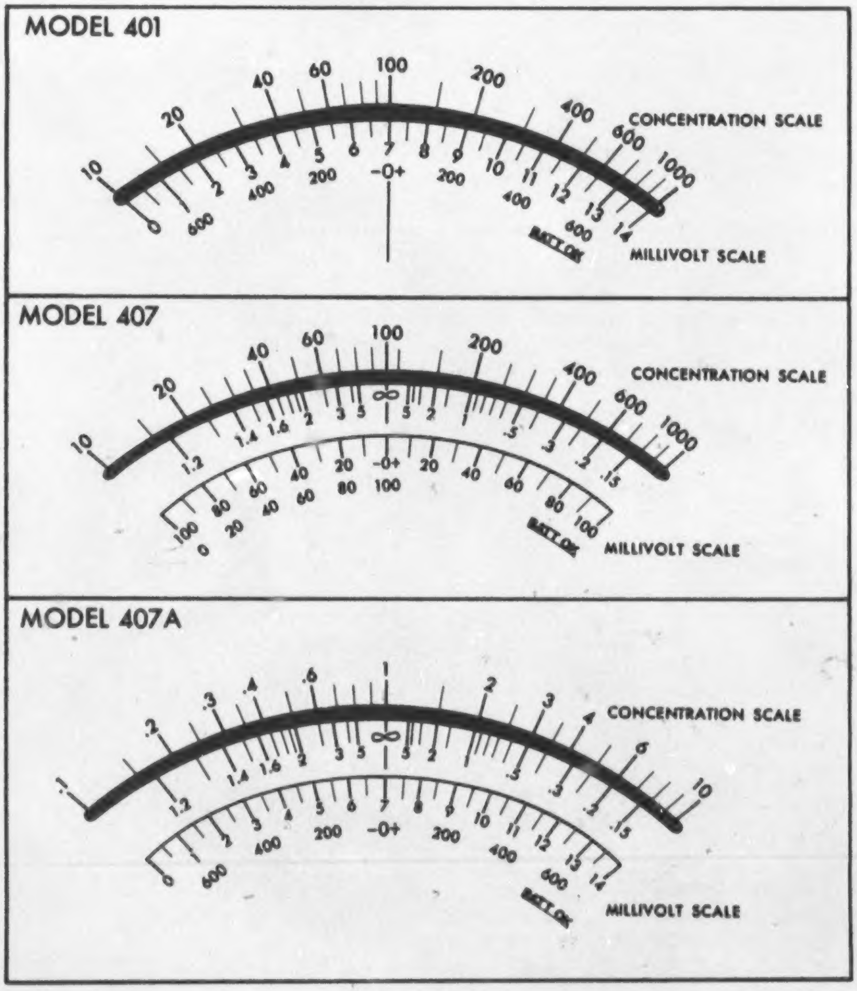

MODEL 401
10
20
40
60
100
200
400
600
1000
CONCENTRATION SCALE
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
600 400 200 -0+ 200 400 600
BATT OK
MILLIVOLT SCALE
MODEL 407
10
20
40
60
100
200
400
600
1000
CONCENTRATION SCALE
1.2 1.4 1.6 2 3 5 ∞ 5 2 1 .5 .3 .2 .15
100 80 60 40 20 -0+ 20 40 60 80 100
0 20 40 60 80 100
BATT OK
MILLIVOLT SCALE
MODEL 407A
.1
.2
.3
.4
.6
1
2
3
4
6
10
CONCENTRATION SCALE
1.2 1.4 1.6 2 3 5 ∞ 5 2 1 .5 .3 .2 .15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
600 400 200 -0+ 200 400 600
BATT OK
MILLIVOLT SCALE

FIGURE 5

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.2.1a	Add a 1 + 1 mixture of ammonia-free distilled water and sodium hydroxide-sodium thiosulfate solution to each Kjeldahl flask to be used. Add glass beads and, using appropriate apparatus, distill 50 ml of this solution. The distillate should be checked to insure that it is ammonia-free. This can be done with the ammonia probe and meter or by use of the Nessler's color reagent.	
F.1.1a	In using a specific ion meter the calibration range is arbitrary within the appropriate range of the method. Should the samples to be run fall outside the range of 0.01 to 1.0 mg NH_3-N/liter, a new range can be set on the instrument. This is done in the same manner as set down in section F but using a tenfold concentration increase. For example, using the 1.0 mg NH_3-N/liter solution in step F.1.1 and a 10 mg NH_3-N/liter solution in step F.2.1 gives a range of 0.1 to 10 mg NH_3-N/liter.	
H.1.1c	If the electorde is accidentally left in the air, rather than in a solution, that portion of the internal filling solution between the inside of the membrane and the sensing element will dry out. To restore the electrode to operation hold the electrode by the outer body and, grasping the electrode cable directly above the cap, pull on the cable so as to lift the sensing element off the membrane. Fresh internal filling solution will now flow under the membrane. The electrode will now be ready for use.	Instruction Manual Ammonia Electrode Model 95-10, Orion Research, Inc., Cambridge, MA 02139.
H.4	Membrane failure is characterized by a shift in electrode potential, drift and poor response. Membrane failure may be apparent on visual inspection as dark spots or discoloration of the membrane. Handling the membrane during installation may adversely affect it and shorten its life. Handle the membrane with the tweezers provided. A membrane will last from one week to several months depending on usage.	Ibid